Niche separation of *Clausocalanus* species in the Mediterranean Sea and in the Atlantic Ocean

Thesis

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Abstract

This thesis represents an integrated study of the ecology and reproductive biology of *Clausocalanus*, a planktonic copepod genus that is widespread in oceanic and coastal regions but still poorly investigated. Principal aim of this study was characterizing the niches of eight congeneric species on the basis of investigations conducted at different space and time scales in the Mediterranean Sea and in the Atlantic Ocean.

The combined results of field surveys and experiments showed that *Clausocalanus* species were characterized by different ecological and reproductive traits. Along ecological gradients of temperature and autotrophic biomass, the niches were clearly defined and separated in small (*C. paululus*, *C. pergens*, and *C. furcatus*) and large (*C. lividus* and *C. mastigophorus*) species, while they largely overlapped in medium-sized species (*C. parapergens*, *C. jobei*, and *C. arcuicornis*).

Clausocalanus presents a unique reproductive feature among planktonic copepods, since its species have two different egg-laying modes: small and medium-sized species are sac-spawners while the large species are broadcast spawners. The first data on specific sex ratio, embryo viability and hatching success were here provided for *Clausocalanus* wild populations. Egg production rates of broadcaster species were more than three times higher than those of sac-spanwers. Embryo viability was low in broadcast spawners and

very high in sac-spawners. Despite the two groups had similar secondary production, broadcast spawners had lower recruitment, which could contribute to their lower abundance at sea. A nuclear vital probe revealed that eggs of successive clutches were fertilized even in the absence of males, suggesting that re-mating is not necessary in this genus.

The results of this thesis highlight the relevance of *Clausocalanus* in marine zooplankton communities and the distinctness of its species, towards a better understanding of niche differentiation among congeners in the epipelagos.

CONTENTS

CHAPTER 1

Introduction

Marine environments cover most of earth surface (≈72%) and occupy a large total volume of about 1.37 10^9 km^3. In all marine regions, the whole water column was successfully colonized by planktonic animals, which occur with large number of individuals and species, most of them concentrated in the epipelagic domain. Even in marine oligotrophic environments, which represent the vast majority of the oceans and where abundances are limited by very low primary production, numerous zooplanktonic species coexist. "*How is it possible for a number of species to coexist in a relatively isotropic or unstructured environment all competing for the same sort of materials?*". This question, phrased by Hutchison in 1961, had arisen by his previous remarks (Hutchinson, 1957) and is known as *the paradox of the plankton* (Hutchinson, 1961). Hutchinson referred to phytoplankton but these issues can actually be applied to animal planktonic communities too.

In the early 20^{th} century, Grinnell (1917) and Elton (1927) independently introduced the niche concept to which they attributed different meanings. While Grinnell focused on the place occupied by a species in the environment, Elton focused on the role of a species in the community. Both Grinnell and Elton's niche definitions included the Darwinian metaphor later named the *competitive exclusion principle* (also known as Gause's principle, Volterra-Gause principle and Lotka-Volterra principle) which states

that "complete competitors cannot coexist", i.e., two species cannot coexist if they require and consume in the same mode and intensity exactly the same resources for living (Hardin, 1960). Hutchinson, redefined the niche as an attribute of the population (or species) in relation to its environment (Hutchinson, 1957) and formalized the niche concept in terms of the occupation of a hypervolume (N-dimensional space) whose dimensions represent each measurable feature of the environment that influences organisms. The niche might be defined as the region of the hypervolume in which the fitness (survival and reproduction) of the individuals is positive. This hypervolume may be called the species' *fundamental niche* (Hutchinson, 1957). Due to competition and other interactions, the species may be excluded from some parts of its fundamental niche. The reduced hypervolume where a species occurs is termed its *realized niche* (Hutchinson, 1957). Hutchinson's definition is static (fixed in space) and atemporal (fixed in time) so does not immediately suggest how to represent variability of organisms in their utilization of the environment over time or variability of species populations in space and time (Griesemer, 1999).

When the temporal and/or spatial scales are taken into account in characterizing species niches, two interpretations can be obtained. According to the environmental niche definition (those of Grinnell and Elton), two species "fill the same niche" in different places or times not only when they are ecologically similar but also when the environmental structures are similar. According to the population niche definition (that of Hutchinson), two species "have similar niches" when they have similar ecological, morphological and behavioral features at that time(s) and/or site(s). These kind of

similarities themselves should be considered in terms of ecological and evolutionary processes, studying habitat characteristics as well as phylogenetic relationships among species (Colwell, 1999) .

MacArthur and Levins (1967) stated that the more ecologically similar two competing species are, the less likely they are to coexist, so there must be some limit to the ecological similarity of coexisting species. The degree of similarity, and thus the level of competition, has been measured as niche overlap in relation to niche breadth, for which many theoretical formulations and empirical measures have been devised and explored (Colwell, 1999 and references therein). The differences between niches of coexisting species are usually considered as the result of their coevolution, which occurred in the past under determined pressure of competition, although almost in every case this hypothesis remains untested (Connell, 1980).

Plankton niche characterization is a challenging issue in pelagic systems, where the environment is apparently unstructured but a high degree of coexistence is observed even among congeneric species. Hutchinson stated that temporal habitat variability does not permit marine communities to reach an equilibrium preventing single species dominance (Hutchinson, 1961). Richerson *et al.* (1970) hypothesized that contemporaneous heterogeneity might be the major diversifying factor with enough persistence to allow many species to exploit the whole habitat simultaneously. According to Ghilarov (1984), neither of the two previous hypotheses can provide an adequate explanation of *the paradox of the plankton* and he suggested an alternative explanation, the "coexistence principle" formulated by Boer (1980). According to the coexistence principle, species that are taxonomical closely related (and

likely also similar in their ecological needs and features) co-occur in the same sites more frequently than could be expected from a random distribution of species.

Phytoplankton, the pelagic primary producers that are food for zooplankton, are unicellular with some species forming colonies, but their lack of complex tri-dimensional structure when compared to land primary producers like grasses and trees (roots, trunk, branches, leafs and their components), limits the number of niches for aquatic primary consumers while it increases enormously in terrestrial primary producers (flora). Moreover, phytoplankton generation times are much shorter than those of terrestrial plants. This implies that short-time variability of the environment as caused by heavy rainfall, stormy days, or hot weather periods can have a pronounced impact on water characteristics, light conditions and nutrient supply, preventing the equilibrium among autotrophic organisms and therefore also in the further steps of the trophic web (Reynolds, 1993). Several studies have attempted to quantify, or at least identify, which factors prevent the equilibrium being reached: seasons and weather (Reynolds, 1993), physical processes (mesoscale vortices and fronts) that generate transport barriers and prevent complete mixing and competitive exclusion (Bracco *et al.*, 2000), extinction and invasion (Huisman *et al.*, 2001); trophic interactions and competition (as well as seasonality, Scheffer *et al.* (2003)). All such studies stress the importance of the scales in niche characterization in pelagic systems, differing from hours to years and from centimeters to kilometers. The issue is efficiently expressed by Reynolds' statement "one summer in plankton dynamics is comparable to many centuries of terrestrial succession" (Reynolds,

1993).

Coexistence of zooplankton species has been extensively studied, mainly in cladocerans in freshwater environments (e.g., *Daphnia* spp., Leibold and Tessier, 1997). In marine environments, most zooplankton are represented by copepods (Arthropoda, Crustacea), which are considered the "insects of the seas". In comparison with insects, copepods have much lower diversity but are much more abundant due to the large volume of seawater on earth (Schminke, 2007). Copepods successfully colonized the whole water column and numerically dominate the zooplankton communities in a very large variety of ecological conditions (Huys and Boxshall, 1991). Up to now, 2258 species of pelagic copepods have been inventoried in the world's oceans (Razouls *et al.*, 2005-2007). The order Calanoida (43 families) is the most numerically abundant (80.4 % of total copepod numbers)(Razouls *et al.*, 2005-2007). The large number of copepod genera co-occurring in a certain depth layer, coupled with the occurrence of as many as 10 to 15 congeneric species (Mauchline, 1998), challenge the niche definition for this group.

In copepod communities, large overlap of species niches were observed in the North Pacific by Hayward and McGowan (1979). These authors pointed out two main aspects to explain copepod coexistence: the first relates to the reduction of competition through specialization and niche separation, and the second relates to the non-equilibrium in which competition, and the resulting competitive exclusion, is prevented by some disturbing influence such as patchiness, seasonality or predation. Nevertheless, the copepod species recorded had broad depth distributions and a wide variety of food items in their gut contents, which suggests that they were generalists (Hayward and

McGowan, 1979). Persistent co-occurrence of copepod species believed to utilize the same or similar resources was observed by McGowan and Walker (1979), who reported that most of the co-occurring species overlapped in body size and had similar body plans, and some were congeners.

Disturbance-perturbation theory, which gives satisfactory explanations of diversity maintenance in benthic systems, does not apply to a pelagic system such as the central gyre of the North Pacific (McGowan and Walker, 1985). On the other hand, in regions with more pronounced environmental gradients, species might minimise interspecific competition by various means. In the eastern tropical Pacific Ocean, species may reside in different positions in the water column (Longhurst, 1985). In the North Atlantic Ocean, species may have seasonal displacement of their main reproductive periods, occupy different trophic levels, have different reproductive strategies, or different migratory behavior throughout the year (Williams, 1988).

According to Darwin (1872), struggle will generally be more severe between congeneric species than between species of distinct genera because of their generally remarkable similarity in morphology and habits. Several studies have focused on the occurrence of congeneric copepod species to look for patterns or mechanisms that should prevent competition among them. Body size and different patterns of vertical migration between highly stratified water masses were the two mechanisms postulated to allow copepod congeneric species coexistence in the Gulf of Guinea (Bainbridge, 1972). Some congeneric species show vertical habitat-partitioning (e.g., family Scolecitrichidae Kuriyana and Nishida, 2006) while others largely overlap (e.g., 12 euchaetid species, Mauchline, 1995) or do not separate in the vertical domain

(e.g., 5 *Pleuromamma* species, Haury, 1988). Some congeneric species show clear differentiation in their distribution along spatial gradients (e.g., 4 *Acartia* species in estuarine environment, Alcaraz, 1983), shifts in the temporal patterns of the breeding season (e.g., six *Acartia* species, Lakkis, 1994) or no shift (e.g., *Centropages*, *Oithona*, *Haloptilus* and *Pleuromamma* pairs of congeneric species, McGowan and Walker, 1979). Different geographical distribution and different size of feeding appendages have been suggested for five *Euchirella* species (von Vaupel Klein, 1997). Among small planktonic copepods, consistent patterns of ontogenic and diel vertical distribution shifts were observed in *Paracalanus, Acartia* and *Oithona* in the Pacific Ocean (Ueda, 1987). Böttger-Schnack (1996) and Paffenhöfer and Mazzocchi (2003) recorded different vertical distribution of congeneric species in small copepods like *Oncaea*, *Oithona*, *Paracalanus*, *Calocalanus*, and *Clausocalanus*.

1.1. *Clausocalanus*: the target copepod genus

Recent studies have highlighted the importance of small planktonic marine copepods ($\leq$ 1 mm in length) in terms of abundance, biomass and grazing impact in marine environments (Morales *et al.*, 1991; Roman *et al.*, 1993; Calbet *et al.*, 2001; Hopcroft *et al.*, 2001; Gallienne and Robins, 2001; Turner, 2004). Among the small planktonic copepods, the genus *Clausocalanus* is one of the most common and abundant genera in oceanic environments, especially in subtropical-tropical regions

In their precious revision on the morphology and biometry of the species

that represent genus *Clausocalanus*, Frost and Fleminger also reported the geographical distribution of the species, which is summarized briefly here. The genus *Clausocalanus* has a world wide distribution. Eight species are circumglobal (*C. mastigophorus*, *C. lividus*, *C. parapergens*, *C. arcuicornis*, *C. jobei*, *C. furcatus*, *C. pergens* and *C. paululus*), two species are restricted to the Indo-Pacific Oceans (*C. farrani* and *C. minor*), while the last three species have circumaustral distribution (*C. ingens*, *C. laticeps* and *C. brevipes*). The distribution ranges of many *Clausocalanus* species frequently overlap (Frost and Fleminger, 1968).

Clausocalanus species are small, most of them are $\leq$1 mm in total length (TL), and the largest ones do not exceed 2 mm (Frost and Fleminger, 1968). Females are larger than males: the largest female is that of *C. ingens* (TL_{max} 1.90 mm) while the largest male is that of *C. lividus* (TL_{max} 1.45 mm); the smallest female and male belong to *C. paululus* (TL_{min} 0.66 mm and 0.47 mm, respectively). In the Mediterranean Sea, eight *Clausocalanus* species are reported (Razouls and Durand, 1991) out of the thirteen species described for this genus (Frost and Fleminger, 1968)(Figure 1.1).

Since the revision of Frost and Fleminger (1968), *Clausocalanus* species have been the object of only a few detailed studies on their ecology (Table 1.1) and biology (Table 1.2).

Only recently, it has been highlighted that *Clausocalanus* presents an unusual reproductive feature among planktonic copepods, i.e. congeneric species have different spawning modes. Saiz and Calbet (1999) reported that *C. lividus* releases eggs freely in the water whereas *C. arcuicornis* carries its eggs as was observed in *C. furcatus* by Mazzocchi and Paffenhöfer (1998)

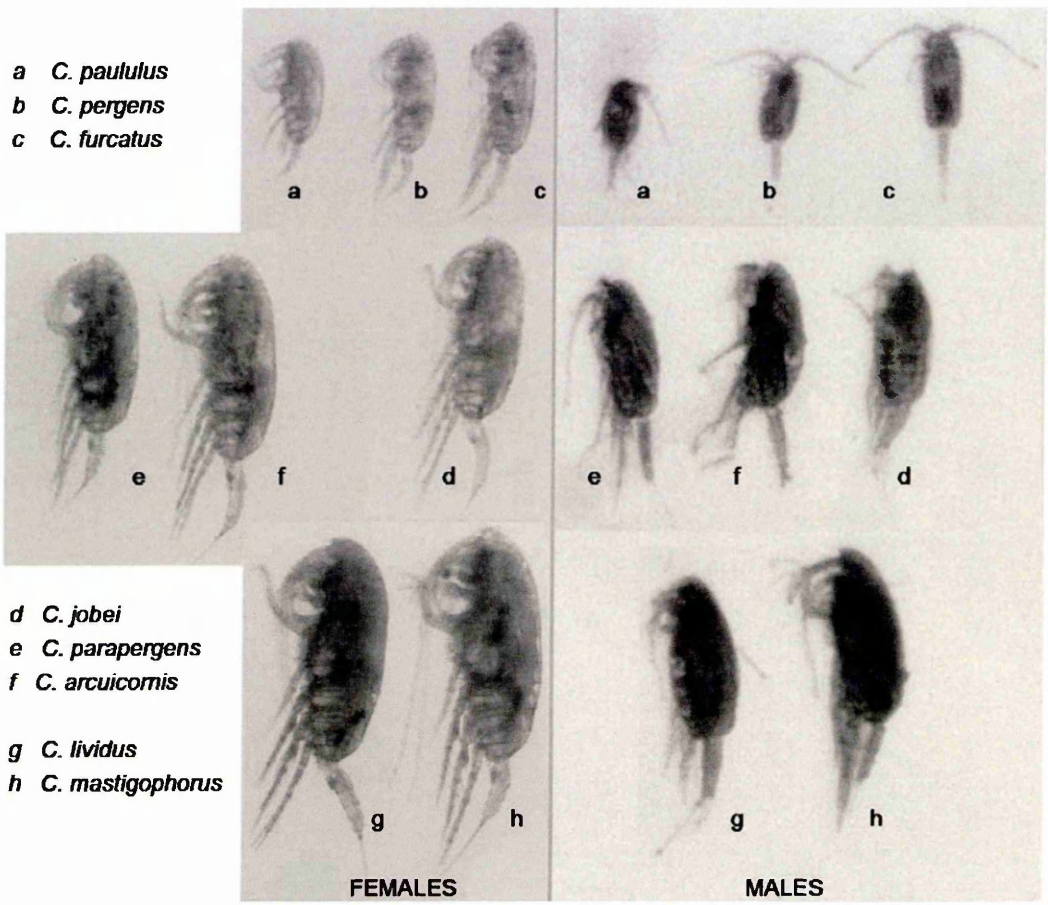


Figure 1.1. *Clausocalanus* species females (left) and males (right) occurring in the Mediterranean Sea.

and in *C. paululus*, *C. furcatus*, *C. jobei*, *C. minor* and *C. farrani* observed in fixed samples by Frost (1969).

Clausocalanus also differs from other small calanoids in its particular swimming behaviour (Mazzocchi and Paffenhöfer, 1999). The motion pattern of *C. furcatus* is characterized by continuous and very convoluted small loops performed at very high speed (10 body lengths per second on average, Mazzocchi and Paffenhöfer, 1999). Similar swimming behaviour has been

also observed in other *Clausocalanus* species (Mazzocchi and Paffenhöfer, 1999, personal observation).

In synthesis, most *Clausocalanus* species are very abundant in oceanic regions and some of them are numerically important in both eutrophic and oligotrophic environments. *Clausocalanus* species span over a relatively narrow size range (0.66-1.90 mm) and many of them overlap in their biogeographical distribution (Frost and Fleminger, 1968)(Table 1.3). Differently from other copepod genera, *Clausocalanus* shows two different reproductive traits (broadcast vs. sac spawners) and peculiar swimming behavior. All together, these features make *Clausocalanus* a very interesting target for the study of the niche concept in planktonic congeneric species.

Table 1.1. Studies on *Clausocalanus* distribution published after the revision of Frost and Fleminger (1968) and here grouped by topic. arc *C. arcuicornis*, bre *C. brevipes*, far *C. farrani*, fur *C. furcatus*, job *C. jobei*, ing *C. ingens*, lat *C. laticeps*, liv *C. lividus*, mas *C. mastigophorus*, min *C. minor*, par *C. parapergens*, pau *C. paululus*, per *C. pergens*.

Year	Authority	Area	arc	bre	far	fur	job	ing	lat	liv	mas	min	par	pau	per
Distribution															
1969	Frost	Global ocean	x	x	x	x	x	x	x	x	x	x	x	x	x
1979	Salman and Brenning	Atlantic	x			x	x			x				x	x
1980	Björnberg	Atlantic	x	x		x	x	x	x		x		x	x	x
1991	Mazzocchi and Ianora	Atlantic		x				x	x						
1991	Razouls and Durand	Mediter.	x			x	x			x	x		x	x	x
1994	Zunini Sertorio and Licandro	Mediter.	x			x									
1997	Errhif et al	Pacific		x					x					x	
2002	Hsieh and Chiu	Pacific	x	x		x		x			x	x	x		x
2002	Peterson and Keister	Pacific	x										x	x	x
2004	Siokou-Frangou et al.	Mediter.	x			x	x							x	x
2006	Isari et al.	Mediter.	x			x	x			x	x		x	x	x
Seasonal distribution															
1969	Carli and Cristafi	Mediter.	x			x	x			x	x		x	x	x
1970	Hure and Scotto di Carlo	Mediter.	x			x	x			x	x		x	x	x
1975	Williams and Wallace	Atlantic	x			x	x			x	x		x		x
1976	Regner	Mediter.	x			x	x			x	x		x	x	x
1979	Álvarez-Marques	Atlantic	x			x	x			x				x	x
1980	Hure et al.	Mediter.	x			x	x			x	x		x	x	x
1995	Mazzocchi and Ribera d'Alcalà	Mediter.	x			x								x	x
1996	Siokou-Frangou	Mediter.	x			x	x			x				x	x
1998	Siokou-Frangou et al.	Mediter.	x			x	x			x				x	x
2003	Chiba and Saino	Pacific													x
2004	Peralba and Mazzocchi	Mediter.	x			x	x			x	x		x	x	x

Table 1.1 (Continued)

Year	Authority	Area	arc	bre	far	fur	job	ing	lat	liv	mas	min	par	pau	per
Vertical distribution															
1969	Frost	Pacific	x		x	x	x		x	x	x	x	x	x	x
1970	Hure and Scotto di Carlo	Mediter.	x			x	x			x	x		x	x	x
1980	Hure et al.	Mediter.	x			x	x			x	x		x	x	x
1984	Scotto di Carlo, et al.	Mediter.	x			x	x			x	x		x	x	x
1985	Longhurst	Pacific	x			x								x	
1986	Schulz	Atlantic	x			x					x		x		
1990	Fragopoulu and Lykakis	Mediter.	x			x	x							x	x
1992	Pancucci-Papadopoulou et al.	Mediter.	x			x	x			x	x		x	x	x
2001	Fragopoulu et al.	Mediter.	x			x	x			x	x		x	x	x
2003	Paffenhöfer and Mazzocchi	Atlantic				x					x				x
2004	Peralba and Mazzocchi	Mediter.	x			x	x			x	x		x	x	x
Diel vertical migration															
1969	Frost	Pacific	x		x	x	x		x	x	x	x	x	x	x
1971	Champalbert	Mediter.	x			x									
1977	Timonin.	Atlantic	*Clausocalanus* spp.												
1990	Fragopoulu and Lykakis	Mediter.	x			x	x							x	x
1992	Checkley et al.	Pacific				x									
1994	Zunini Sertorio and Licandro	Mediter.	x			x									
1996	Atkinson et al.	SubAnt.							x						
1999	Vega-Pérez et al.	Atlantic				x									
2003	Hwang et al.	Pacific	x			x					x				
2004	Lo et al.	Pacific	x		x	x		x			x	x	x		

Table 1.2. Studies on *Clausocalanus* biology published after the revision of Frost and Fleminger (1968).

Year	Authority	Aspect	Species
1971	Heron and Bowman	Morphology of postnaupliar stages	*C. brevipes, laticeps*
1972	Björnberg	Morphology of naupliar stages (NI-NV)	*C. furcatus*
1973	Nival and Nival	Mandible intersetule distance	*C. arcuicornis*
1977	Arashkevich	Digestion duration	*C. mastigophorus*
1984	Mayzaud et al.	Gut fluorescence	*C. arcuicornis*
1987	Sazhina	Egg production	*C. furcatus*
1990	Ayukai	Fecal pellet production	*C. arcuicornis*
1994a,b	Landry et al.	Field gut pigment contents	*C. arcuicornis, pergens*
1995	Roff et al.	Bacterivory and growth rates of nauplii and copepodids	*C. furcatus*
1995	Webber and Roff	Annual biomass and egg production	*C. furcatus*
1996	Atkinson et al.	Relationship between vertical migration, gut fullness and gut evacuation rate	*C. laticeps*
1998	Mazzocchi and Paffenhöfer	Reproduction in laboratory conditions	*C. furcatus*
1999	Mazzocchi and Paffenhöfer	Swimming and feeding behaviour	*C. furcatus*
1999	Saiz and Calbet	Reproduction	*C. arcuicornis, furcatus, lividus*
2002a	Mayzaud et al.	Respiration rates	*C. laticeps*
2002b	Mayzaud et al.	Ingestion rates on natural particle assemblages	*C. laticeps*
2003	Bucklin et al.	Phylogeny	All species
2003	Montresor et al.	Grazing rates on dinoflagellate cysts	*C. lividus*
2004	Buttino et al.	Hatching success	*C. furcatus*
2005	Bi	Population dynamics	*C. furcatus*
2005	Cornils	Grazing and reproduction in natural particle assemblages	*C. farrani, furcatus*
2005	Wiggert et al.	Grazing success modeling	*C. furcatus*
2006	Bi and Benfield	Population dynamics	*C. furcatus*
2006a	Paffenhöfer et al.	Oxygen consumption	*C. furcatus*
2006b	Paffenhöfer et al.	Ingestion rates on natural particle assemblages	*C. furcatus*
2006	Uttieri	Antennulae morphology and sensory structures	*C. furcatus*
2007	Blanco-Bercial and Álbarez-Marqués	RFLP for species discrimination	*C. arcuicornis, jobei, lividus, pergens*
2007	Cornils et al.	Reproduction in natural particle assemblages	*C. farrani, C. furcatus*

Table 1.3. *Clausocalanus* species ordered by size categories used in this thesis. Females and males size ranges and distribution are those reported by Frost and Fleminger (1968). Spawning modes that are reported in the literature (+ Giesbercht, 1892; * Frost, 1969; - Saiz and Calbet, 1999), are personal observations (!), or are personal hypotheses (?).

Species	Size category	Range (mm) Females	Males	Distribution	Spawning mode
C. paululus	Small	0.66-0.80	0.47-0.56	Circumglobal	S*
C. pergens	Small	0.70-1.10	0.52-0.67	Circumglobal	S!
C. furcatus	Small	0.94-1.31	0.70-0.92	Circumglobal	S*
C. jobei	Medium	1.01-1.56	0.87-1.07	Circumglobal	S*
C. parapergens	Medium	0.97-1.38	0.97-1.14	Circumglobal	S!
C. farrani	Medium	1.04-1.20	0.87-0.99	Indo-Pacific Ocean	S*
C. minor	Medium	1.08-1.26	0.79-1.04	Indo-Pacific Ocean	S*
C. arcuicornis	Medium	1.15-1.62	0.97-1.17	Circumglobal	S+
C. lividus	Large	1.26-1.77	1.13-1.45	Circumglobal	B-
C. mastigophorus	Large	1.23-1.84	1.05-1.45	Circumglobal	B!
C. brevipes	Large	1.24-1.62	1.12-1.27	Circumaustral	B?
C. laticeps	Large	1.25-1.67	1.01-1.10	Circumaustral	B?
C. ingens	Large	1.44-1.90	0.99-1.08	Circumaustral	B!

1.2. Aims and approaches

The significance of genus *Clausocalanus* is not only due to its numerical importance over wide range of trophic conditions in the ocean but also due to the presence of unique biological features (see Section 1.1). However, difficulties in taxonomic identification and problems in rearing these copepods in laboratory conditions (Mazzocchi and Paffenhöfer, 1998) have limited the number of studies on the ecology and biology of *Clausocalanus* at the species level.

The abundance and diffusion of *Clausocalanus* species in the epipelagic domain have prompted me to investigate aspects of their ecology and reproductive biology, in order to improve our understand of the reasons for their success in various marine regions.

This thesis has been focused on eight out of the thirteen described species of *Clausocalanus*, and it has in particular been aimed at; 1) determining the extent of the niche separation among congeneric species, and 2) understanding whether their reproductive traits might account for their population distribution and success. These issues have been addressed through; 1) the study of *Clausocalanus* occurrence in very different marine regions both in the Mediterranean Sea and in the Atlantic Ocean, and 2) the acquisition of reproductive parameters in wild populations.

The ecological and biological approaches have been developed, respectively, in Chapters 2-5 and Chapter 6 of the thesis. Thereafter, in Chapter 7 the quantitative information acquired for both the ecology and reproductive biology of the species have been combined to depict an overall scenario where the eight *Clausocalanus* species niches were described and the extent of their niche separation was evaluated.

The first approach (Chapters 2-5) towards the niche characterization of *Clau-*

socalanus species consisted in depicting their ecological features by investigating their occurrence at different temporal and spatial scales.

The seasonal and vertical distributions of *Clausocalanus* species in epipelagic oligotrophic waters were analysed in 2002 at an offshore site (Stn L20) in the open Gulf of Naples (Tyrrhenian Sea, Western Mediterranean). That annual cycle was compared with the annual cycle conducted at the coastal eutrophic Stn MC that is located in the inner Gulf of Naples and is the site of a long-term zooplankton time-series since 1984. Such comparison allowed the study of seasonal distribution of *Clausocalanus* species under different trophic conditions (Chapter 2).

The distribution and composition of spring *Clausocalanus* assemblages were investigated during two oceanographic surveys conducted in the Eastern and in the Western Mediterranean, respectively in the oligotrophic Ionian Sea (spring 2002) and in the eutrophic North Balearic Sea (spring 2003). This allowed me to compare *Clausocalanus* assemblages in the open sea in the same season but in very different trophic conditions (Chapter 3).

The distribution of *Clausocalanus* species on a large spatial scale (latitudinal) was addressed in the Atlantic Ocean during the Atlantic Meridional Transect programme (AMT 15, September-October 2005)(Chapter 4).

The whole data set of *Clausocalanus* occurrence during the above mentioned surveys was analysed in relation to the principal environmental parameters recorded in parallel to zooplankton sampling (temperature and autotrophic food availability) in order to characterize the realized ecological niches of *Clausocalanus* species (Chapter 5). The environmental and the population niche concept previously introduced have been taken into account when approaching the niche characterization of *Clausocalanus* species. The species realized niches were determined from the observed distributions in relation to environmental gradients and with respect

to their coexisting congeners.

The second approach (Chapter 6) consisted of the acquisition of data on reproductive parameters in eight *Clausocalanus* species (*C. pergens*, *C. furcatus*, *C. jobei*, *C. parapergens*, *C. arcuicornis*, *C. lividus*, *C. mastigophorus*, and *C. ingens*) through a number of onboard experiments conducted within the same projects where the study of *Clausocalanus* distribution was addressed, in the Mediterranean Sea and in the Atlantic Ocean. Such investigations provided new data on field fecundity rates and hatching success. Species reproductive activity has been also analyzed in relation to environmental gradients and inter-species comparison has been analyzed through weight specific fecundity rates corrected to a single temperature.

Data of abundance and fecundity rates have allowed estimations of secondary production of some *Clausocalanus* species in the Mediterranean Sea and in the Atlantic Ocean.

Finally, the major results of this thesis have been combined to depict a general scenario (Chapter 7) where *Clausocalanus* niches have been discussed in terms of body size and egg-laying modes.

Throughout the thesis, *Clausocalanus* species are always presented according to the mean body size reported by Frost and Fleminger (1968), from the smallest (*C. paululus*) to the largest (*C. mastigophorus*).

CHAPTER 2

Seasonal and vertical distribution in the Gulf of Naples (Tyrrhenian Sea)

Clausocalanus has been often reported as an important genus in both coastal and offshore zooplanktonic communities, but only a few studies have analysed occurrence and abundance at the species level (Table 1.1). Coastal and offshore areas differ for many aspects of physical processes and trophic conditions, the former being richer in food due to major nutrient supply from different sources (upwelling and runoff, mainly). In the Gulf of Naples, both coastal and offshore systems can be recognized (Carrada *et al.*, 1980) and eutrophic and oligotrophic conditions can be easily compared. The Gulf of Naples is a SW oriented bay with an average depth of 170 m over an area of approximately 870 km^2 (Figure 2.1); the narrow eutrophic coastal system is under the influence of land runoff while the offshore system has oligotrophic characteristics typical of the Tyrrhenian Sea (Carrada *et al.*, 1980). Strong gradients in nutrient and chlorophyll concentrations are generated by land runoff and are recorded only in the littoral area. The exchange between coastal and offshore waters is restricted to the very nearshore environment due to the characteristic physiography and bottom topography of the Gulf (Carrada *et al.*, 1980). The location and width of the boundary between the two systems are variable over the seasons (Carrada *et al.*, 1980; Marino *et al.*,

1984) and highly dynamic water mass distribution may enhance the exchange between them (Casotti *et al.*, 2000).

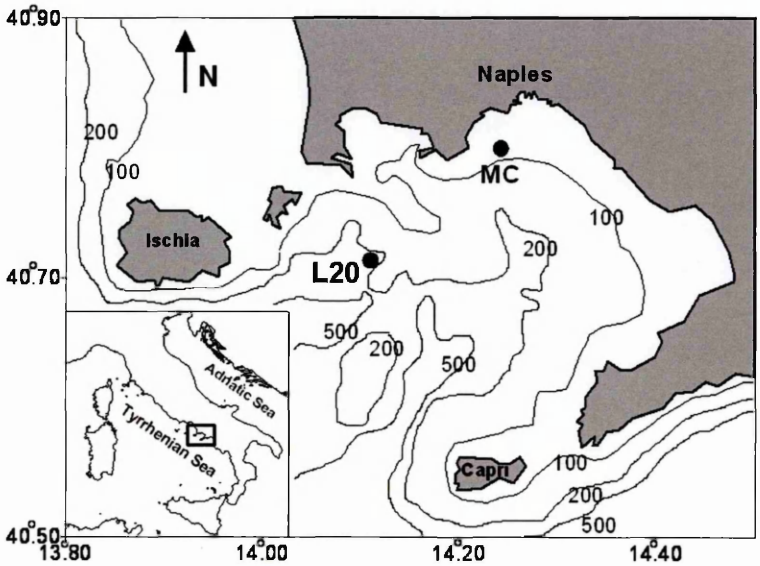


Figure 2.1. Map of the Gulf of Naples with the stations (L20 and MC) surveyed during this study.

The study of the seasonal and vertical distribution of *Clausocalanus* species in oligotrophic conditions was conducted at the offshore Stn L20, which is permanently influenced by oligotrophic waters and only occasionally by eutrophic coastal waters (Carrada *et al.*, 1980). In winter, it is occupied by an homogeneous water column of Tyrrhenian Intermediate Water (TIW; 14°C, 38 psu) as a result of convective mixing. During spring and summer, this water warms and remains above 50 m depth becoming the Tyrrhenian Surface Water (TSW; 14-26°C, 38 psu), while the core of TIW remains between 50 and 100 m until late autumn, when it is incorporated into the new production of TIW (Carrada *et al.*, 1980).

In the inner part of the Gulf, a long-term time-series is being conducted since 1984 at Stn MC. This area is characterized by high temporal and spatial variability for physical and chemical parameters (Ribera d'Alcalà *et al.*, 1989) while recurrent

patterns in the occurrence of several phytoplankton (Zingone and Sarno, 2001), ciliate (Modigh, 2001) and zooplankton (Mazzocchi and Ribera d'Alcala, 1995) species have been recorded. The annual sampling cycles conducted in parallel at Stn L20 and Stn MC were compared for the analysis of *Clausocalanus* assemblages in two different trophic systems.

Part of this chapter has been published (see Peralba and Mazzocchi, 2004).

2.1. Material and methods

Annual cycle at the offshore Stn L20

Stn L20 is located in the open Gulf of Naples (40°41'N, 14°15'E; >300 m depth)(Figure 2.1). Zooplankton sampling was carried out from February 2002 to February 2003 at monthly frequency (biweekly when possible), on board the R/V *Vettoria*. Sampling was missed in September 2002.

On each sampling occasion a Seabird CTD equipped with a SCUFA® submersible fluorometer was deployed to record environmental parameters (temperature, salinity and fluorescence). Water samples were then collected at discrete depths by Niskin bottles for Chl *a* measurements and SCUFA® calibration. Four liters of sea water from each Niskin bottle were filtered on board through Whatman GF/F filters and frozen at -80°C for further HPLC (high-performance liquid chromatography) analyses. Fluorescence data were linearly fitted with Chl *a* concentrations measured in parallel from Niskin bottles by HPLC. Each fluorescence unit (f.u.) corresponded to 0.91 μg Chl *a* L^{-1} according to the regression equation: $y = 1.10x$ (r^2=0.71), after excluding fluorescence profiles recorded on 16 May 2002 and from 26 August to 11 November 2002 because of technical problems with the fluorometer. Since it is well known that the chlorophyll:fluorescence

relationship can be variable depending on phytoplanktonic groups, the calibration was intended to give a valid estimate of the chlorophyll concentration.

Zooplankton samples were collected in the morning in the upper 200 m water column according to the CTD profiles: above and below the thermocline, around the deep chlorophyll maximum (DCM), and in two strata in the homogeneous waters (Table 2.1). In case of a mixed water column, the sampling strata were standard depths (every 50 m, from 200 m to surface).

Table 2.1. Details on samples collected at Stn L20. Collection time is reported in local time that in Italy corresponds to the Coordinated Universal Time (UTC) plus 1 hour. The UTC is a high-precision atomic time standard.

Date	Time (UTC+1)	Sampled layers (m)	Physical structure
14/02/2002	10:10	200-150; 150-100; 100-50; 50-0	Mixed
21/03/2002	10:00	200-150; 150-100; 100-50; 50-0	Mixed
24/04/2002	9:55	200-150; 150-100; 100-50; 50-20; 20-0	Stratified
16/05/2002	11:20	200-150; 150-100; 100-50; 50-30; 30-0	Stratified
19/06/2002	9:50	200-150; 150-100; 100-50; 50-20; 20-0	Stratified
03/07/2002	11:00	200-150; 150-100; 100-50; 50-20; 20-0	Stratified
23/07/2002	9:40	200-150; 150-100; 100-50; 50-20; 20-0	Stratified
06/08/2002	11:00	200-150; 150-100; 100-70; 70-30; 30-0	Stratified
26/08/2002	10:00	200-150; 150-110; 110-80; 80-40; 40-0	Stratified
03/10/2002	10:00	200-150; 150-100; 100-50; 50-0	Mixed
15/10/2002	9:20	200-150; 150-100; 100-50; 50-0	Mixed
12/11/2002	9:00	200-150; 150-100; 100-50; 50-0	Mixed
05/12/2002	9:20	200-150; 150-100; 100-50; 50-0	Mixed
15/12/2002	10:00	200-150; 150-100; 100-50; 50-0	Mixed
20/01/2003	10:30	200-150; 150-100; 100-50; 50-0	Mixed
20/02/2003	10:00	200-150; 150-100; 100-50; 50-0	Mixed

Vertical hauls were performed in each discrete layer with a double WP-2 closing net (200 μm and 70 μm mesh aperture). Once at the surface, the nets were carefully rinsed after each tow and the content of the cod ends were immediately fixed and preserved in a 4% buffered formaldehyde-seawater solution. The vol-

umes of filtered seawater (V) were calculated taking into account the area of the net mouth (A), the difference in winch readings (δL) and the wire angle (α)(V = A x δ L x cosα, m^3). Attention was always paid in maintaining the wire angle below 10°. Filtered volumes ranged from 5 to 12.5 m^3.

Zooplankton samples were analyzed under a stereoscope for taxonomic identification and counts. At least $1/5^{th}$ of the entire sample was analyzed taking repeated aliquots with a large mouth graduated syringe after accurate mixing (modified Stempel pipette method). *Clausocalanus* adults (females and males) were identified to species and sexed according to Frost and Fleminger (1968). Sex ratio was calculated as males/females ratio. Copepodid stages were identified at genus level (mainly stages III-V were retained by the 200 μm mesh). All other copepods were counted without further identification. Abundances are expressed as number of individuals per cubic metre (ind. m^{-3}). Only samples from 200 μm mesh net have been analysed so far and their data are presented here. Selection pattern described by a mathematical model (Nichols and Thompson, 1991) showed that a mesh size of 75% of the copepod carapace width catches ≈95% of the individuals of that size present in the water. Since the carapace width of the smallest adult *Clausocalanus* is larger than 150 μm, it was assumed that the 200 μm mesh net should collect the vast majority of adult *Clausocalanus* individuals present in the seawater.

Qualitative information on *Clausocalanus* reproductive activity was also acquired during sample counts, i.e. number of adult females with attached spermatophore(s) and sac remains at the genital pore. The Reproductive Index (RI) was calculated as the percentage of females with sac remains and/or spermatophores to total counted female. Sac spawners *Clausocalanus* species dislodge the egg mass/sac when are stressed so they are usually lost in fixed samples. Only seldom

females still had the sac remains with few eggs during the counts. *C. lividus* and *C. mastigophorus* are broadcast spawning species so their RI was only based on attached spermatophores. Middle to higher latitude species of copepods usually show seasonality in breeding while copepods living in subtemperate and tropical regions are continuous breeders even though there are periods of higher and lower breeding intensity (Ianora, 1998). Such breeding activity would be reflected in the presence of sexually active males and females. The presence of attached spermatophores represents an evidence of sexually active males and sac remains at the female's genital pore of sexually active females. Further information can be obtained by examining the degree of maturity of the gonads of both males and females, or full spermathecae in the females. Ovarian maturity was attempted to be assessed during this study but the collected information was rejected and considered a too subjective parameter since no information on ovarian development is available for *Clausocalanus* but absence of oocites pigmentation was noticed by Cornils *et al.* (2007) in *C. furcatus* and *C. farrani* and dark and opaque females were related to parasitized females by Ianora *et al.* (1990) in *C. pergens*, *C. furcatus*, *C. arcuicornis* and *C. lividus*. Population information regarding copepodid and nauplii abundances have been used as well to determine species' breeding seasons in several studies (e.g. Shmeleva and Kovalev, 1974), but despite some copepodid stages were collected during this study, they were not identified at species level and so were not useful to characterize *Clausocalanus* breeding seasons at species level. Using only the presence of attached spermatophores and/or remains of eggs-sac as a reproductive index is therefore not complete but has been considered at least indicative of *Clausocalanus* species breeding activity in this study.

PCA analysis were preformed to correlate species abundance, reproductive

index and sex ration along the annual cycle integrated in the upper 200 m and at discrete depth layers.

Annual cycle at the coastal Stn MC

Stn MC is located 2 miles from the coast, in front of Naples city (40°48.5'N, 14°13'E; ≈80 m depth)(Figure 2.1). Since January 1984, it is the site of a long-term study of the pelagic system. Data collected from February 2002 to February 2003 at weekly or biweekly frequency were utilized for the present study.

CTD and fluorescence profiles were obtained with a SBE911 mounted on a Rosette sampler equipped with Niskin bottles (12 l). Total Chl *a* concentrations were determined at selected depths (surface, 2, 5, 10, 20, 40, 60 m) and analyzed with a spectrofluorometer. Mesozooplankton samples were collected with vertical hauls from 50 m to the surface, using a Nansen net (113 cm mouth diameter, 200 μm mesh size). The volume of filtered seawater was 50 m^3 for each sample. Samples were immediately fixed and preserved in 4% formaldehyde-seawater solution. Specimen identification and counts were performed under the stereoscope. *Clausocalanus* females were identified to species while males and juveniles to genus (counts performed by I. di Capua, Laboratory of Bilogical Oceanography, SZN).

The comparison of both sampling series at Stn L20 and Stn MC was based on the monthly average for the upper 50 m layer, except in August when the integrated 0-70 m (06/08/2002) and the upper 40 m (26/08/2002) at Stn L20 were used.

2.2. Results

2.2.1. Seasonal distribution at Stn L20

Environmental parameters

In the 0-200 m water column from February 2002 to February 2003, the depth-averaged values of temperature showed marked seasonality, a typical pattern of temperate regions. Temperature was low in February 2002 (14.4°C), it gradually increased until it reached the annual maximum in early October (16.3°C) then decreased down to the minimum value in February 2003 (14.3°C). Salinity showed only slight variations ranging from 38.06 psu in January and February 2003 to 38.26 psu in early August 2002. Chl *a* concentrations in the upper 10 m were on average 0.11 μg Chl *a* L^{-1} ($\pm$SD 0.06), showing a first peak in winter (February 2002) and a second lower peak in autumn (mid-October)(Fig. 2.2).

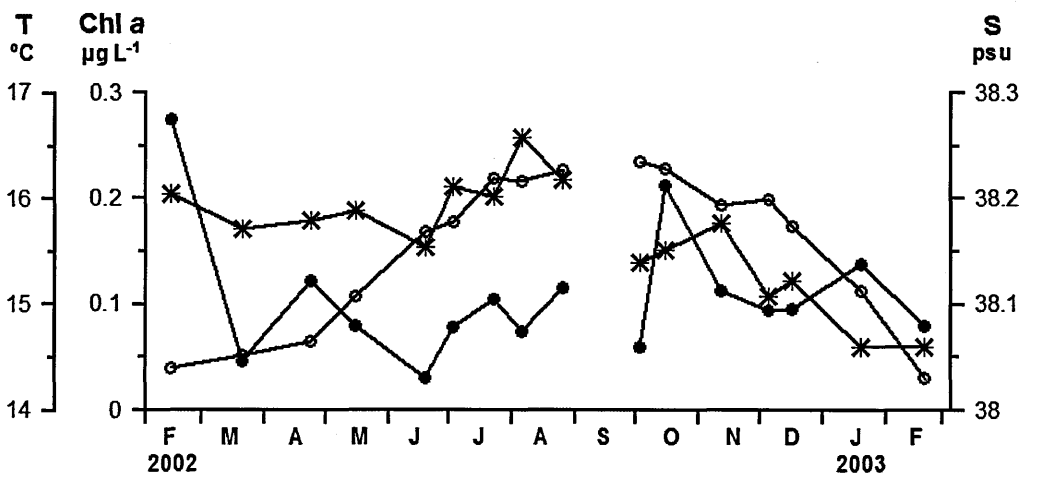


Figure 2.2. Depth-averaged values of temperature (T, open circles) and salinity (S, asterisks) in the upper 200 m, and chlorophyll concentrations (Chl *a*, black circles) in the upper 10 m at Stn L20 from February 2002 to February 2003.

Clausocalanus

In the integrated 0-200 m water column, the abundance of *Clausocalanus* averaged 86.3 ind. m^{-3} ($\pm$SD 60.3) and showed a major peak in April (271.9 ind. m^{-3}) followed by two lower peaks in June and August (Fig. 2.3a). This genus represented on average 25.7% ($\pm$7.4) of total copepod abundance. It was by far the most important genus in winter when the copepod assemblage was diversified (>20 genera) and at its annual minimum, and in early-spring in correspondence with the genus peak abundance (Fig. 2.3a).

Adult females (52.5$\pm$9.4%) and juveniles (39.9$\pm$3.7%) accounted for most of the *Clausocalanus* abundance, while males represented only 7.6% ($\pm$8.1). The contribution of adult females and males was higher in winter and summer and lower in spring and autumn (Fig. 2.3b).

Four species represented all together 89.4% of the adult *Clausocalanus* abundance: *C. paululus* (34.8%), *C. furcatus* (27.7%), *C. arcuicornis* (19.4%), and *C. pergens* (7.5%). They were followed by *C. parapergens* (4.1%), *C. lividus* (2.9%), *C. jobei* (2.6%), and *C. mastigophorus* (1.0%). *C. paululus* prevailed in winter, *C. arcuicornis* in spring, *C. furcatus* in autumn and *C. pergens* contributed more in early-summer (Fig. 2.3c).

In most populations, a temporal succession was observed among peaks in sex ratio (M:F), reproductive index (RI) and adult abundance (Fig. 2.4). *C. paululus* adults peaked in winter (43.6 ind. m^{-3}, February 2002), when other *Clausocalanus* species were nearly absent. The population decreased after April, recovered in early-summer, then decreased gradually until October to increase again in late-autumn and winter (Fig. 2.4a). The highest sex ratio (0.3) occurred in correspondence with adult abundance peak in February 2002 but remained low along the annual cycle. RI occurred in correspondance with adult abundance pattern

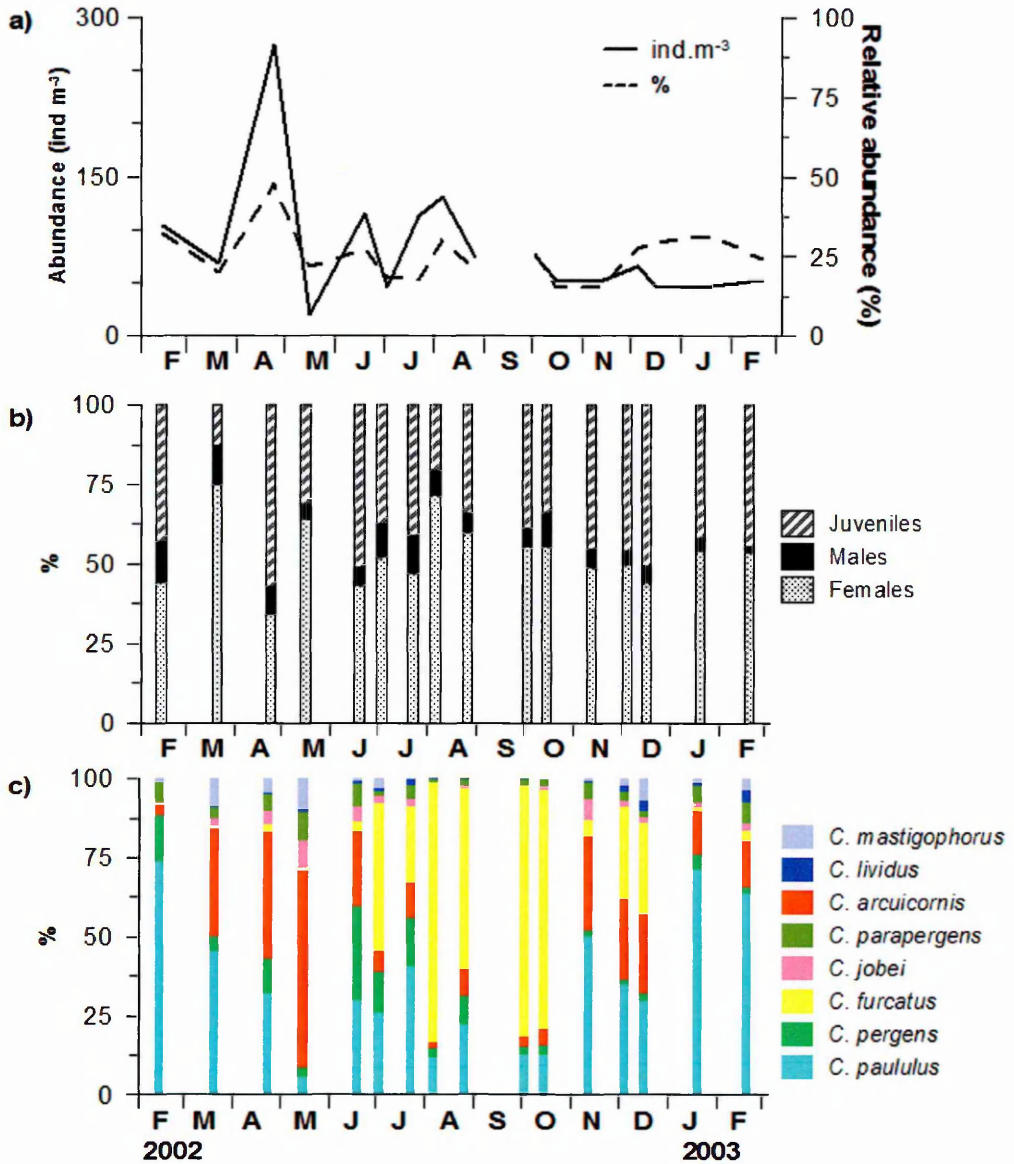


Figure 2.3. *Clausocalanus* occurrence at Stn L20 in the 0-200 m water column. Total *Clausocalanus* abundance (continuous line) and percentage contribution to total copepod abundance (dashed line)(a). *Clausocalanus* population composition (b). Percentage contribution of *Clausocalanus* species to total adult (females and males) abundance of the genus (c).

from March to August (Fig. 2.4a). PCA analysis revealed strong seasonal correlation among adult abundance and reproductive index and weaker but positive correlation with sex ratio.

C. pergens adults had successive peaks in February, April, June (max. 16.8 ind. m^{-3}) and July alternated with low abundances, and very scarce presence during the rest of the annual cycle. Sex ratio was highest in March (0.4), July (0.6) and October (0.5), without correspondence with RI except in October (Fig. 2.4b). PCA analysis confirmed lack of correlation between the variables which were alternated in time.

C. furcatus adults were nearly absent in winter and spring but showed an acute peak in August and high abundances through October (max. in early-August, 85.8 ind. m^{-3}). Sex ratio was high during the phase preceeding the annual peak (0.2-0.5) but also in winter at low adult abundance. RI was generally low, with small increase in correspondence with increasing sex ratio, independently of adult abundance. The highest RI was observed in March (Fig. 2.4c). PCA analysis confirmed seasonal correlation among sex ratio and reproductive index which were negatively correlated with population abundances.

C. jobei adults showed low abundances with a peak in April (5.5 ind. m^{-3}) then gradually decreased. Sex ratio peaked in March (0.2). RI was low during most of the year, with two major peaks in March (before the annual peak of adult abundance) and in August (in correspondence with very low abundance)(Fig. 2.4d). PCA analysis revealed seasonal correlation among sex ratio and reproductive index which were no correlated with population abundances.

C. parapergens adults showed abundance values and patterns similar to *C. jobei*. Sex ratio showed peak values (0.5-0.7) in periods of low adult numbers while the RI was in phase with the abundance pattern from March to August

(Fig. 2.4e). PCA analysis revealed seasonal correlation among the reproductive index and population abundance but these were not correlated with sex ratio.

C. arcuicornis adults showed seasonal patterns very similar to those displayed by *C. jobei* and *C. parapergens* adults but with much higher numbers. Adults reached a peak in April (47.2 ind. m^{-3}) then decreased rapidly and occurred in low abundance throughout the year. High sex ratios were recorded in April (0.5), July (0.7) and October (0.3). RI was quite high (>20%) during most of the year, with three peaks in correspodence with peaks in sex ratio (Fig. 2.4f). PCA analysis revealed seasonal correlation among these three variables.

C. lividus was mainly present from winter to spring, when it reached the highest abundance in March-April (5 ind. m^{-3}). The highest sex ratio in February preceded the annual peak of adult abundance; in that sampling occasion, males were 3.7 times more abundant than females and their number was also high in April (1.2). Very high sex ratio was also recorded in November (3.5) in correspondence with minimum adult abundance. RI was high only in April, in correspondence with peaks of abundance and sex ratio (Fig. 2.4g). PCA analysis revealed negative seasonal correlation among population abundance and sex ratio and no correlation of none of these variable with the reproductive index.

C. mastigophorus, the least abundant species of the genus, occurred in negligible numbers during the whole year. Sex ratio was high only in March and July, when males were slightly more abundant than females (1.3), the peak in July overlapped with peak of RI (Fig. 2.4h). PCA analysis revealed seasonal correlation among sex ratio and reproductive index but these were negatively correlated with population abundance.

In synthesis, all eight *Clausocalanus* species were always present at Stn L20 during the annual cycle (except *C. mastigophorus* in February 2002). The three

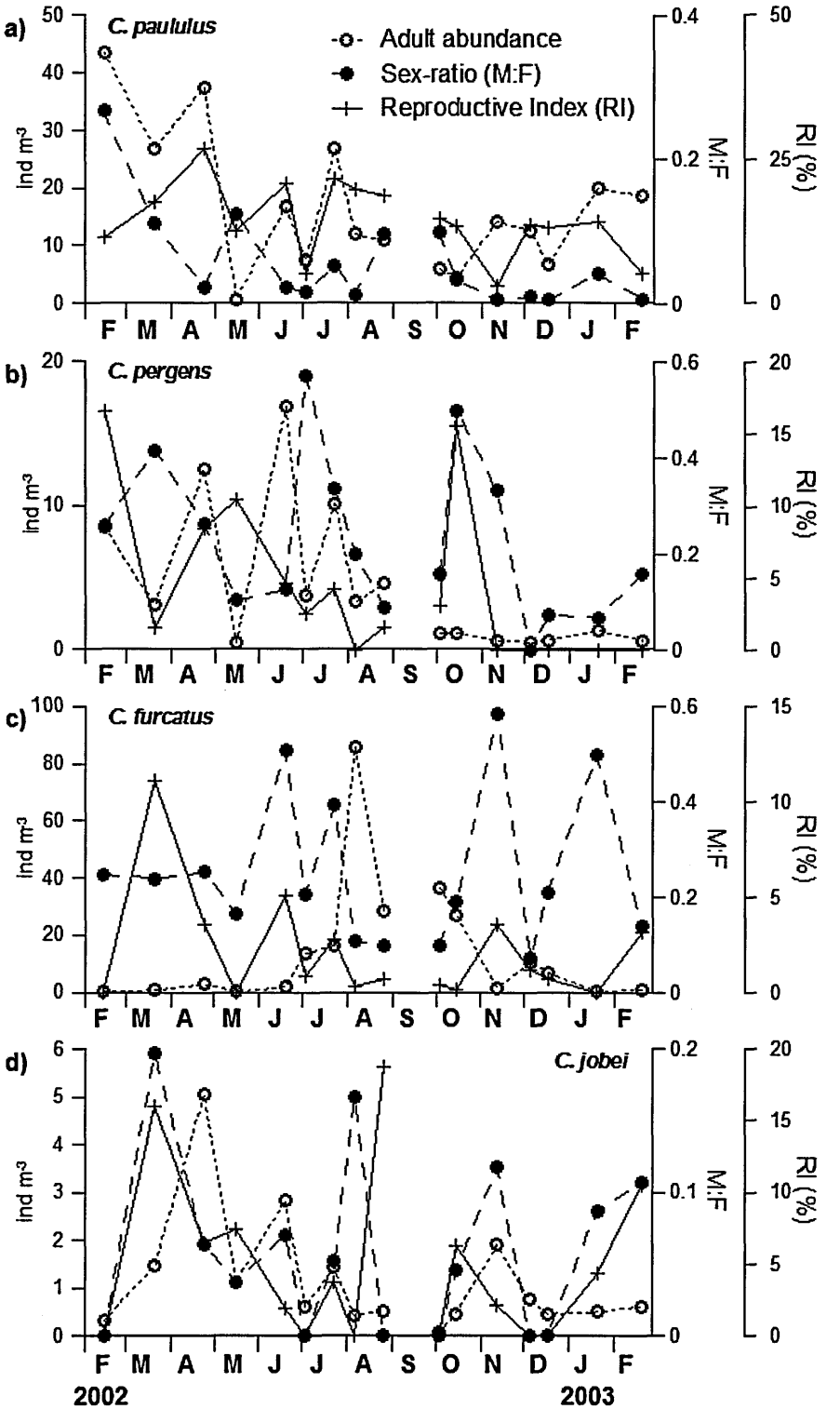


Figure 2.4. Annual cycle of *Clausocalanus* species adult abundance (open circles), sex ratio (M:F)(black circles) and reproductive index (RI)(dashed line) in the upper 200 m at Stn L20. Note different scales among species.

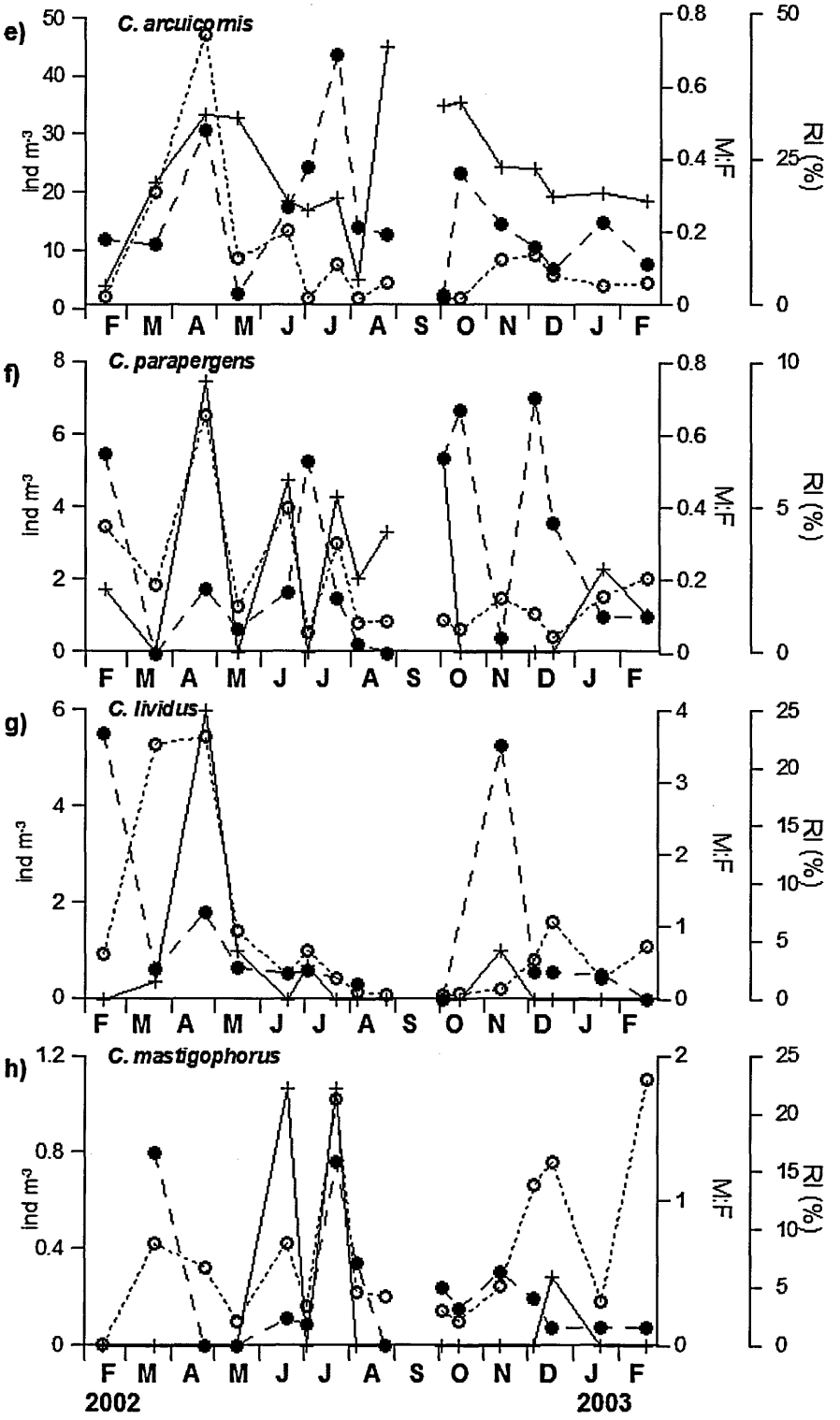


Figure 2.4 (Continued)

most abundant species, *C. paululus*, *C. furcatus* and *C. arcuicornis*, showed successive seasonal peaks, *C. paululus* in winter, *C. arcuicornis* in spring and *C. furcatus* in summer. During the rest of the year, the three species occurred in low abundance. The much less abundant species *C. pergens*, *C. jobei*, *C. parapergens*, and *C. lividus* occurred mainly in spring-early summer. Only *C. mastigophorus* always occurred in negligible numbers and a seasonal cycle could not be discerned.

Sex ratio (males/females) was always lower than 1 (maximum 0.7 in *C. arcuicornis* and *C. parapergens*) in sac spawners and occasionally higher than 1 (more males than females) only in broadcast species (maximum 3.7 in *C. lividus* and 1.3 in *C. mastigophorus*). Despite *C. lividus* and *C. jobei* had similar mean adult abundance, *C. lividus* showed nine fold higher sex ratio (Table 2.2).

The reproductive index remained high throughout the annual cycle in *C. paululus* and *C. arcuicornis*, while it was much lower and variable in the other species (Table 2.3). *C. paululus* had the highest percentage of females carrying spermatophores, while *C. arcuicornis* had by far the highest percentage of females carrying sac remains. All species, except *C. mastigophorus*, were frequently observed carrying more than one spermatophore (Table 2.4).

Table 2.2. *Clausocalanus* gender abundances (ind. m^{-3}) and males/females sex-ratio (M:F). Annual mean, SD and range in the 0-200 m water column at Stn L20 from February 2002 to February 2003.

Species		Mean (±SD)	Range
C. paululus	F	15.3 (10.5)	0.6-36.6
	M	1.1 (2.3)	0.0-9.2
	M:F	0.1 (0.1)	0.0-0.3
C. pergens	F	3.5 (4.2)	0.4-14.9
	M	0.8 (0.9)	0.0-2.6
	M:F	0.2 (0.2)	0.0-0.6
C. furcatus	F	12.7 (20.1)	0.1-77.3
	M	2.3 (4.0)	0.0-8.4
	M:F	0.3 (0.2)	0.1-0.6
C. jobei	F	1.1 (1.2)	0.1-4.7
	M	0.1 (0.1)	0.0-0.3
	M:F	0.1 (0.1)	0.0-0.2
C. parapergens	F	1.6 (1.4)	0.3-5.5
	M	0.3 (0.4)	0.0-1.2
	M:F	0.3 (0.3)	0.0-0.7
C. arcuicornis	F	6.9 (7.9)	1.2-31.9
	M	1.9 (3.7)	0.0-15.3
	M:F	0.2 (0.2)	0.0-0.7
C. lividus	F	0.7 (1.0)	0.0-3.7
	M	0.5 (0.8)	0.0-3.0
	M:F	0.9 (1.2)	0.0-3.7
C. mastigophorus	F	0.3 (0.3)	0.0-1.0
	M	0.1 (0.1)	0.0-0.3
	M:F	0.4 (0.4)	0.0-1.3

Table 2.3. Reproductive activity of *Clausocalanus* species throughout the annual cycle at Stn L20 integrated in the water column (0-200 m). Percentage of females carrying spermatophores, percentage of females presenting sac remains, and total reproductive index (RI). For each component, mean and standard deviation, range of values and the percentage of 16-dates in which reproductive activity was observed (% dates) are reported.

	% females with spermatophore			% females with sac remains			Reproductive Index		
Species	Mean (SD)	Range	% dates	Mean (SD)	Range	% dates.	Mean (SD)	Range	% dates
C. paululus	14.4 (6.5)	3.1-26.9	100	0.1 (0.2)	0-0.6	37.5	14.5 (6.4)	3.1-26.9	100
C. pergens	1.6 (4.0)	0-16.1	37.5	2.7 (3.9)	0-12.5	56.3	4.3 (5.6)	0-16.7	62.5
C. furcatus	1.6 (2.8)	0-11.1	75.0	0.5 (1.1)	0-3.4	43.8	2.1 (2.9)	0-11.1	81.3
C. jobei	2.3 (3.9)	0-12.5	31.3	2.6 (3.4)	0-10.5	50.0	4.8 (5.9)	0-18.8	62.5
C. parapergens	1.4 (1.6)	0-4.2	50.0	1.1 (2.2)	0-6.7	25.0	2.5 (3.0)	0-9.4	56.3
C. arcuicornis	11.4 (7.3)	0.6-28.3	100	11.2 (9.7)	0-34.0	93.8	22.6 (10.9)	3.2-44.2	100
C. lividus	10.3 (27.8)	0-100	31.3	-			10.3 (27.8)	0-100	31.3
C. mastigophorus	3.4 (7.8)	0-22.2	18.8	-			3.4 (7.8)	0-22.2	18.8

Table 2.4. Percentage of observed females that were not carrying a spermatophore and percentage of females carrying one or more spermatophores of those observed carrying spermatophore(s).

	Number of attached spermatophores										
Species	**None**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**11**
C. paululus	84.4	70.4	19.4	5.6	2.4	1.1	0.5	0.2	0.1	0.2	0.1
C. pergens	98.0	60.6	18.2	9.1	12.1						
C. furcatus	99.4	74.1	3.7	3.7	3.7		3.7	3.7	7.4		
C. jobei	99.97	92.9						7.1			
C. parapergens	99.98	58.9	23.5	17.6							
C. arcuicornis	84.1	82.0	13.7	3.0	0.9	0.2	0.2				
C. lividus	95.2	85.8	7.1	7.1							
C. mastigophorus	96.2	100									

2.2.2. Vertical distribution at Stn L20

Environmental parameters

The water column was well mixed in winter 2002. Water temperature in February 2002 ranged from 14.0°C at 200 m to 14.5°C at the surface while salinity was nearconstant in the upper 150 m (around 38 psu) and increased by 0.4 psu below this depth (Fig. 2.5a). The thermocline started to develop in late March in the upper 20 m. Temperature above the thermocline increased rapidly, reaching maximum values (25.3°C) in late July and early August in the upper 20 m and the thermocline was established in April. In autumn, the thermocline slowly deepened throughout the season, reaching 70 m in early December. In winter 2003, the water column was again completely mixed.

Salinity profiles showed maximum values at depth (38.0-38.6 psu at 150-200 m) and lowest values above the thermocline (37.7-38.1 psu)(Fig. 2.5a).

Fluorescence profiles and Chl *a* measurements indicated higher autotrophic biomass in the upper 200 m in February 2002 (0.25 μg Chl *a* L^{-1}). A deep chlorophyll maximum (DCM) was present between 50 and 110 m from March to October. It deepened from March to June, remained at depth during July (0.2 μg Chl *a* L^{-1} at 80 m) and early August, then it rised until early-October (0.1 μg Chl *a* L^{-1} at 70 m)(Fig. 2.5b).

Clausocalanus

Vertical distribution of *Clausocalanus* assemblage showed that copepodids were concentrated in the upper 50 m during most of the year, except in early summer, when they were concentrated deeper, in the DCM layer (Fig. 2.6a). Adults had similar patterns of vertical distribution as copepodids, but with a general tendency to occupy also the deepest layers (Fig. 2.6b, c). Males occurred

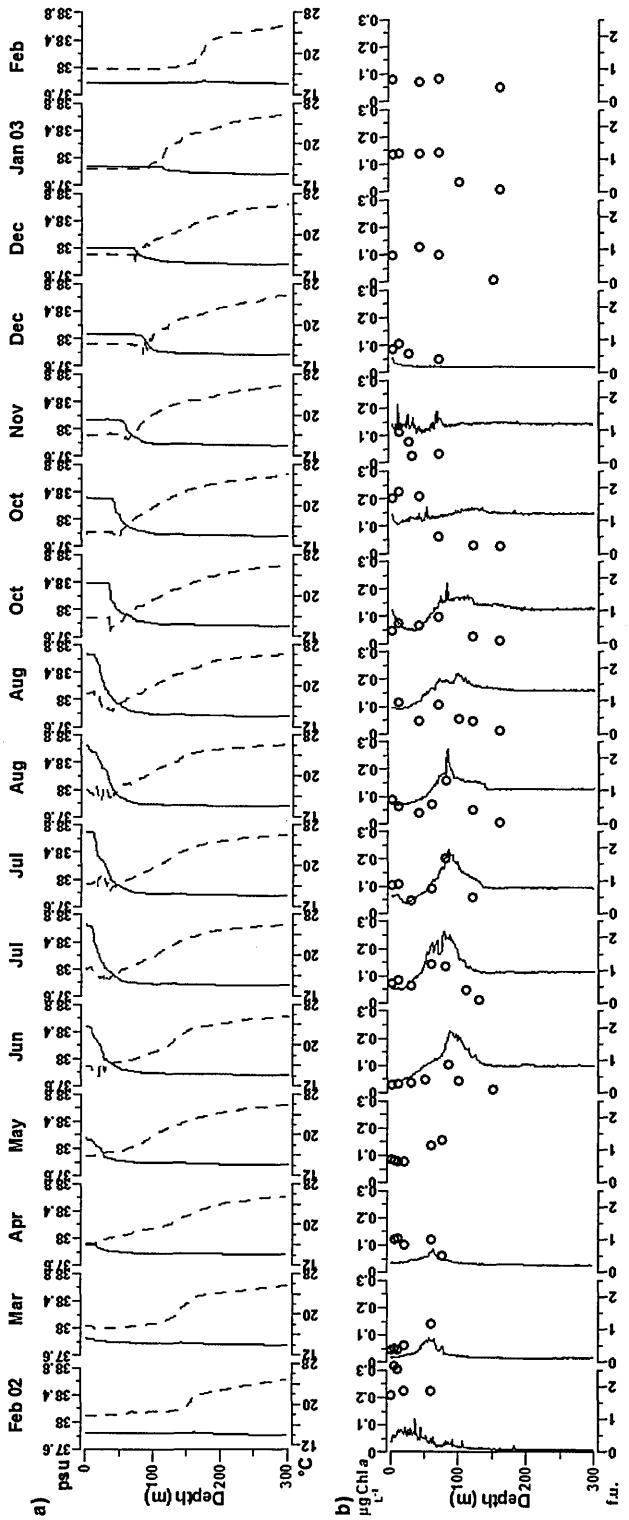


Figure 2.5. Vertical profiles along the water column at Stn L20: (a) temperature (simple line) and salinity (discontinuous line), (b) fluorescence (simple line) and Chl *a* concentrations (open circles).

deeper than females in winter (Fig. 2.6 c).

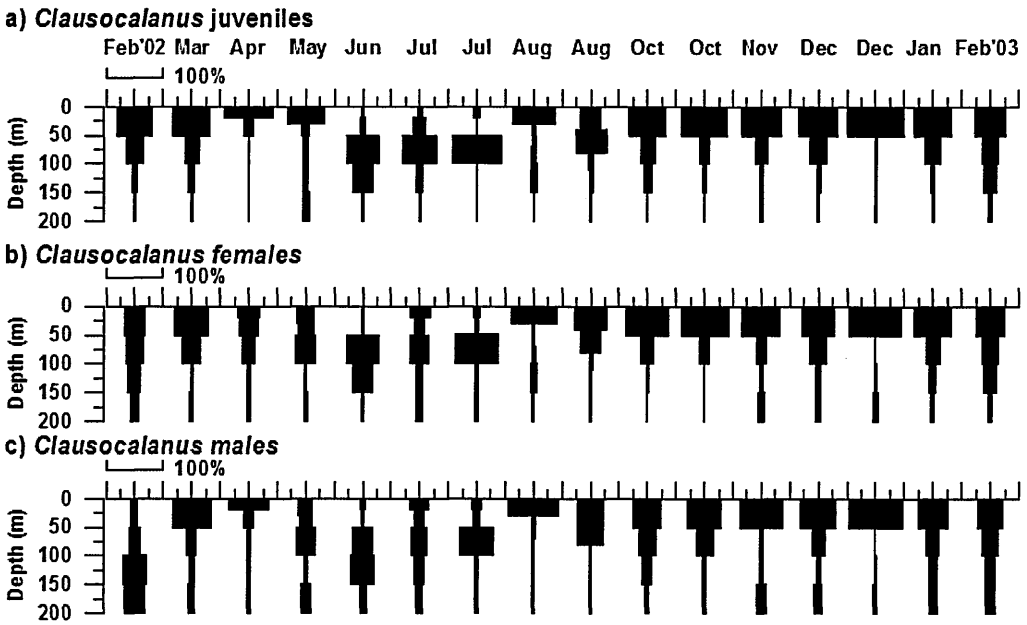


Figure 2.6. Vertical distribution of *Clausocalanus* copepodids (a) and females (b) and males (c) at Stn L20. The relative contribution (%) to total abundance in the water column is shown for each depth layer.

At the species level, *C. paululus*, *C. pergens*, *C. jobei*, *C. parapergens* and *C. arcuicornis* avoided the upper 25-50 m layer from June to mid-October (Fig. 2.7). *C. paululus* (Fig. 2.7a) and *C. pergens* (Fig. 2.7b) were uniformly distributed in the water column in winter, concentrated in the upper 100 m in spring, and occurred below 50 m depth from June to mid-October. Afterwards, they spread again uniformly throughout the water column. Males of these species were recorded deeper than females in winter and on some occasions in summer. *C. paululus* females carrying full spermatophores occurred in the whole water column during the whole annual cycle, independently of male relative percentages (Fig. 2.8a). In contrast, in *C. pergens* only a few females mainly located in the 100-150 m layer carried spermatophores (Fig. 2.8b)

C. jobei (Fig. 2.7d), *C. parapergens* (Fig. 2.7e) and *C. arcuicornis* (Fig. 2.7f),

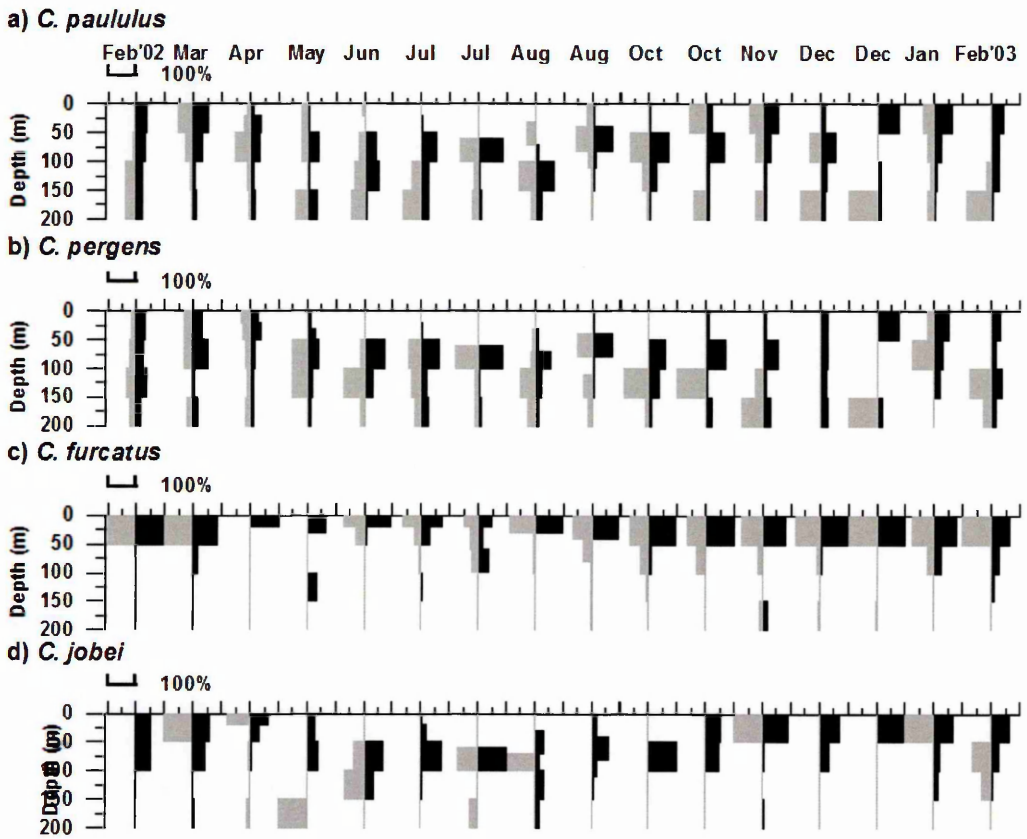


Figure 2.7. Stn L20. Gender vertical distribution of *Clausocalanus* species in the epipelagic water column from February 2002 to February 2003. The relative contributions (%) to total abundance of females (black) and males (grey) in the water column, respectively, are shown for each depth layer.

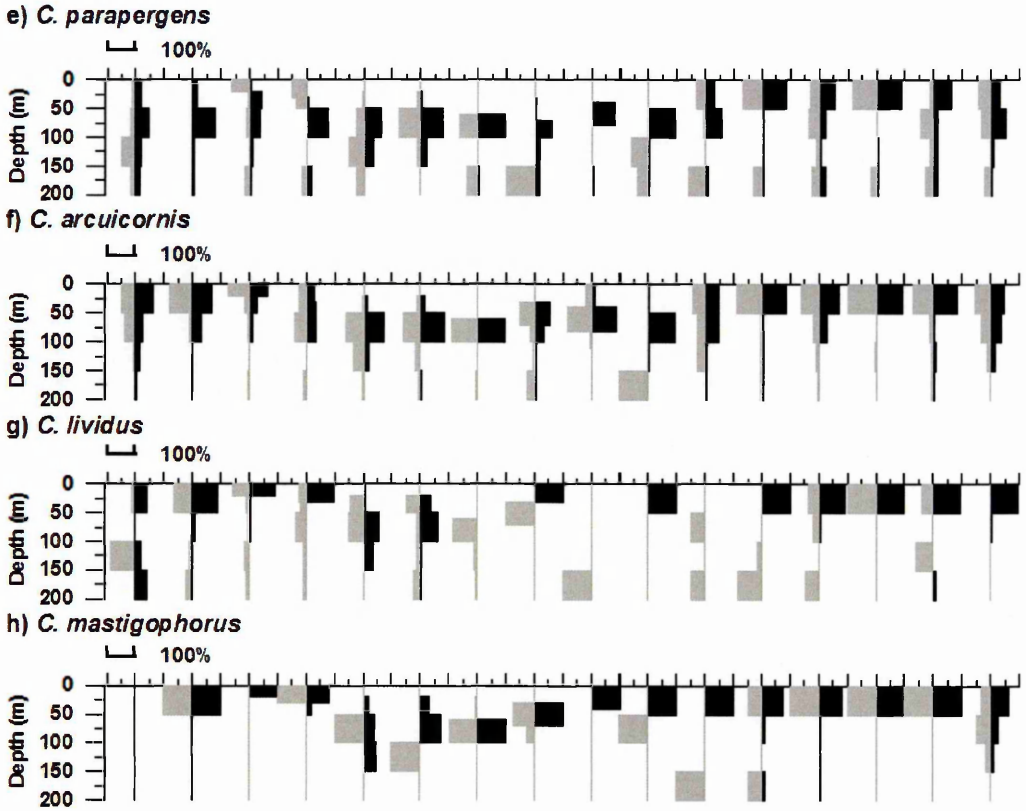


Figure 2.7 (Continued)

showed similar vertical distributions. They were present in the whole water column in winter but they rose to the upper 100 m layer in early-spring. In April, *C. jobei* and *C. arcuicornis* were concentrated in the upper 20 m layer, while *C. parapergens* was uniformly distributed in the upper 100 m. In summer all three species were concentrated below 50 m (Fig. 2.5b) until mid-autumn. In autumn their populations rose again to the upper layer. *C. arcuicornis* females carrying spermatophores were observed through the whole water column in winter and spring, while in summer they occurred above and below the layer where the population was concentrated (Fig. 2.8f).

Unlike the above mentioned species, *C. furcatus* was almost exclusively confined to the upper 50 m (Fig. 2.7c) during the whole annual cycle, except in late

July when it was present down to 100 m. Both females and males had the same depth occurrence. A few females carrying spermatophores were only observed below 50 m in March and July (Fig. 2.8c).

C. lividus showed a variable vertical distribution (Fig. 2.7g). It occurred in the upper layer from winter to spring, and in deeper layers in summer (Fig. 2.5b). In March, females carrying spermatophores were recorded in the 50-100 m layer despite the bulk of the population occurred in the upper 50 m (Fig. 2.8g). *C. mastigophorus* was recorded in the upper layer during most of the year (Fig. 2.7h). It descended to the 50-150 m layer only in summer (June-July) but in August it started to rise again to the upper layer. Females carrying spermatophores were only observed in June (Fig. 2.8h).

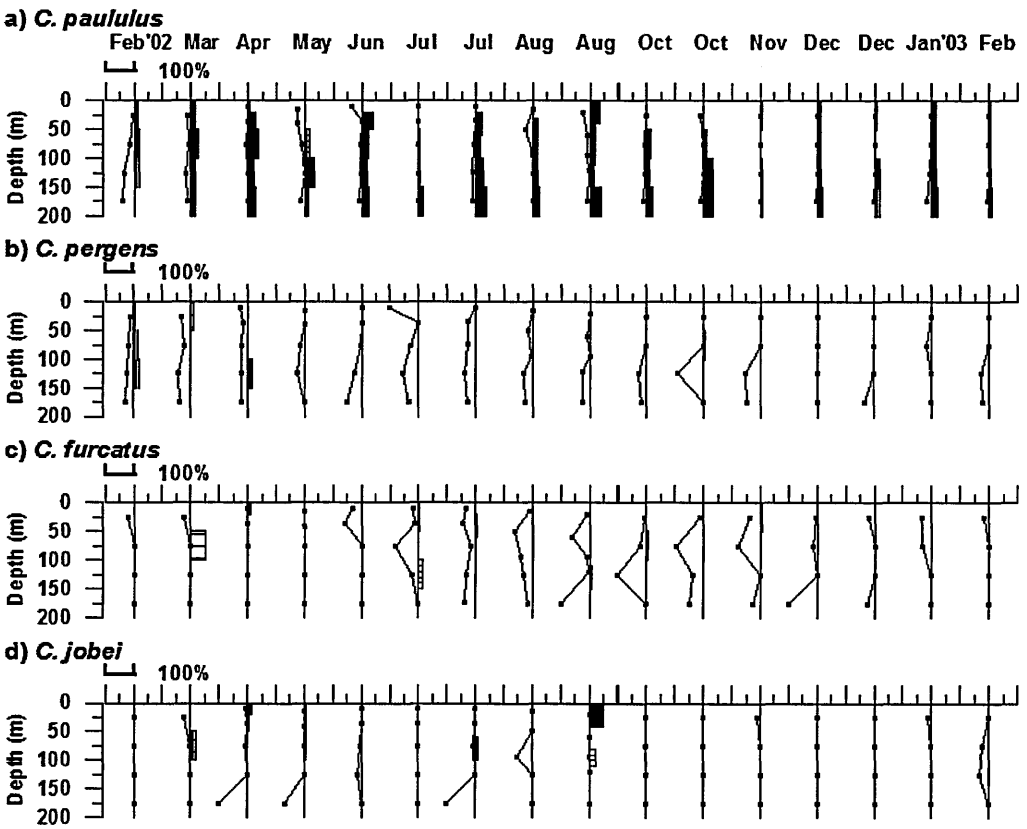


Figure 2.8. Stn L20. Vertical distribution of parameters indicating reproductive activity in *Clausocalanus* species in the epipelagic water column. At each sampling date, the following parameters are shown: contribution (%) of males to total adult abundance (left; continuous line) and contribution (%) of females carrying spermatophores to total female abundance (right; white histograms represent females carrying empty spermatophores while black histograms represent females carrying filled spermatophores).

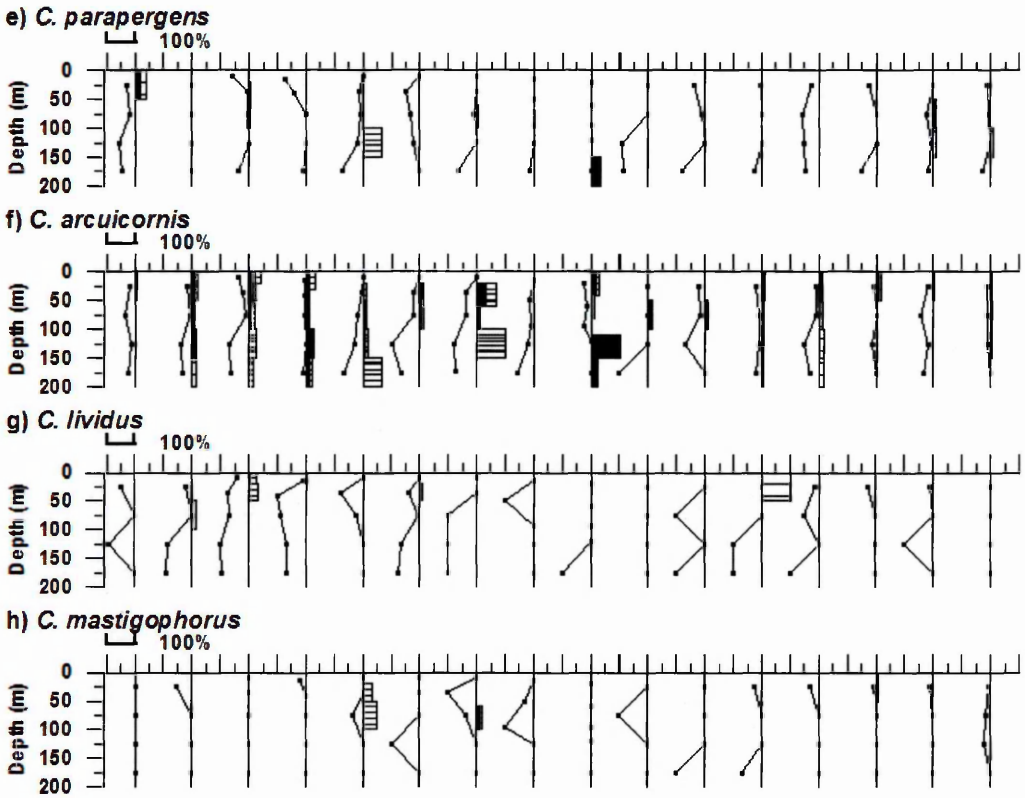


Figure 2.8 (Continued)

2.2.3. Offshore / coastal comparison

The seasonal distribution of *Clausocalanus* females at the species level in the upper 50 m of the water column at Stn L20 during February 2002-February 2003 was compared with that observed at the coastal Stn MC during the same period.

The annual patterns of temperature were very similar at the two sites, with only slightly faster increase in temperature in spring and slower decrease in winter in coastal waters, where the summer peak was steeper than offshore (Fig. 2.9a).

Salinity was slightly higher at Stn L20 than at Stn MC but it displayed similar patterns at both stations with much higher values in winter 2002 than in winter 2003(Fig. 2.9b).

The two sites differed in concentration of autotrophic biomass (Fig. 2.9c). Chl *a* concentration in the upper 10 m was from 1.1 times (February '03) to 13.3 times (March) higher at Stn MC than at Stn L20. In coastal waters, Chl *a* showed two similar peaks (1.46 μg Chl *a* L^{-1}) in spring and autumn. During summer, the lowest concentrations were recorded at both sites, with the minimum value recorded in August (0.03 μg Chl *a* L^{-1}).

The comparison of the *Clausocalanus* annual cycle in offshore and coastal waters in the Gulf of Naples is represented in Fig. 2.10 and highlights the following characteristics:

- very similar abundances in January-March;
- offshore peak in April that was absent in coastal waters;
- slightly higher abundances in coastal waters in May-July and quite higher abundances offshore in August-December.

Clausocalanus relative contribution to the total copepod assemblage was higher at the offshore station especially from February 2002 through April (Fig. 2.10b).

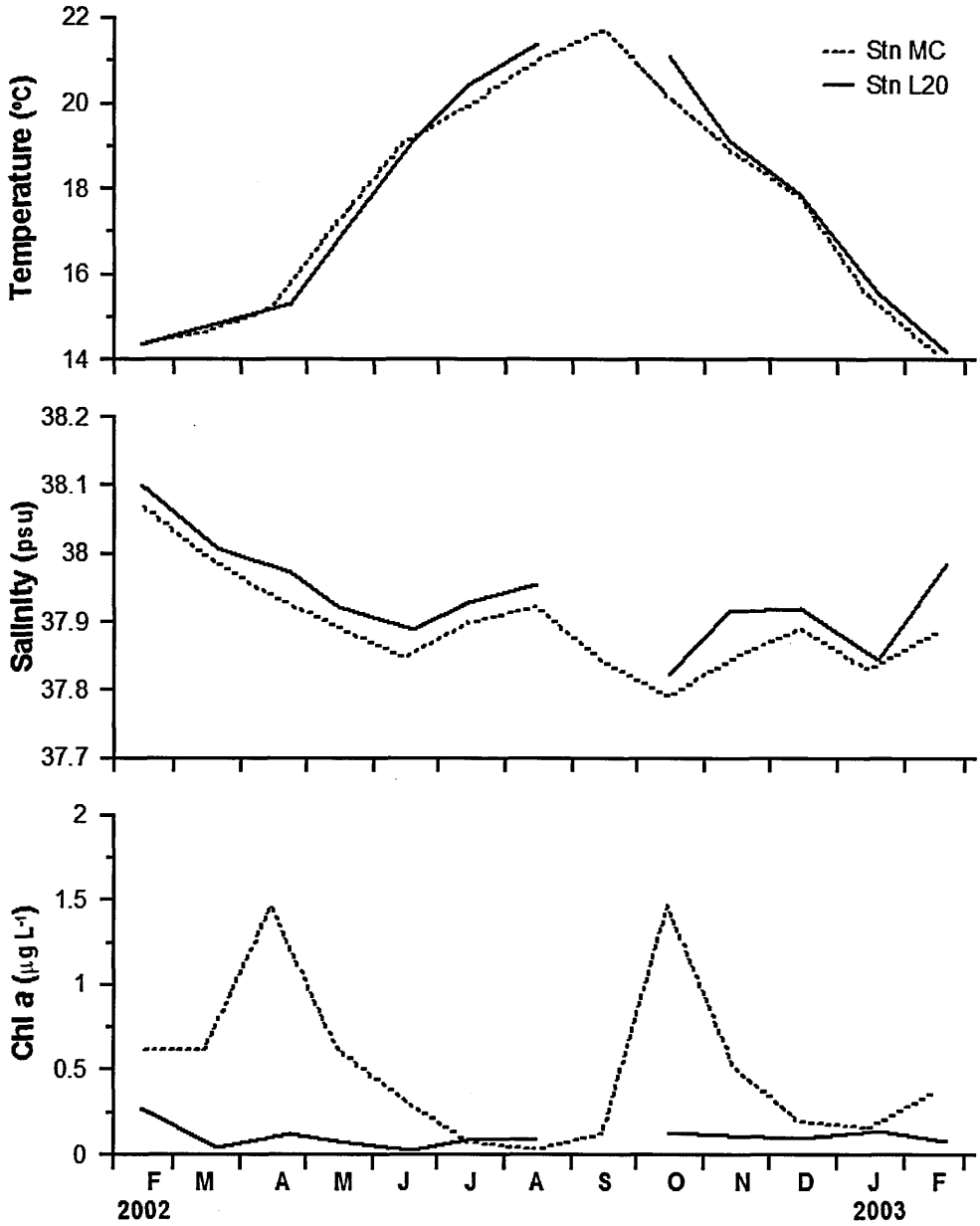


Figure 2.9. Environmental parameters in the 0-50 m depth layer at the offshore Stn L20 (continuous line) and at the coastal Stn MC (dotted line) during February 2002-February 2003. Monthly averages. Stn L20 was not sampled in September.

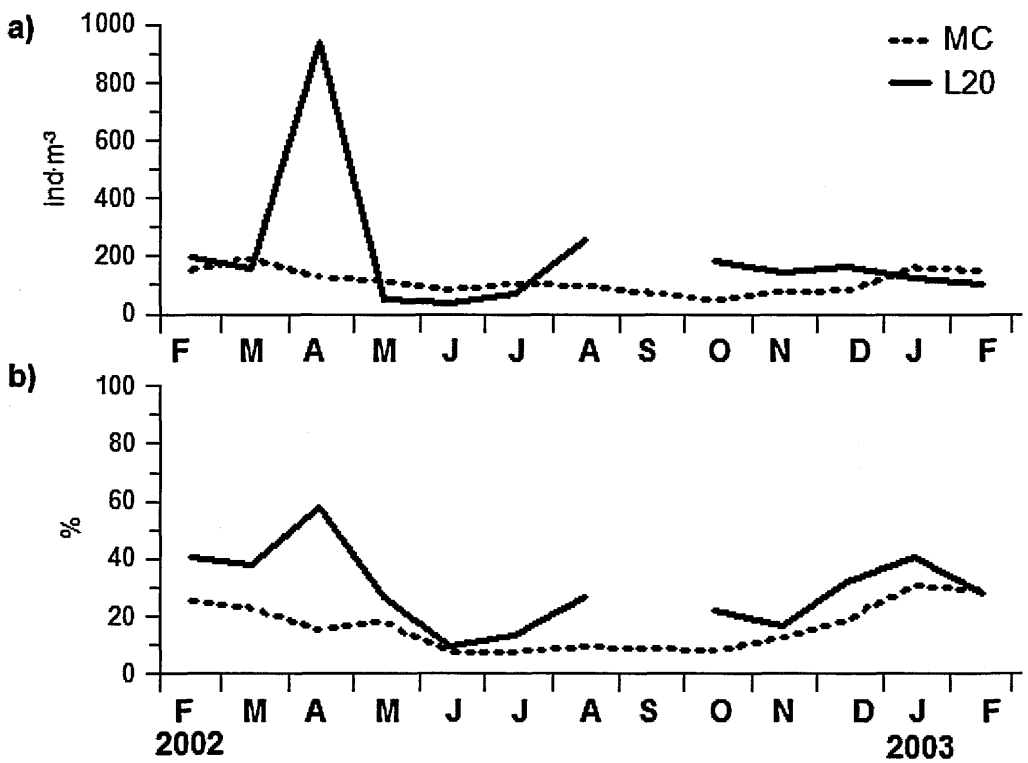


Figure 2.10. *Clausocalanus* adults and copepodids abundance (a) and relative contribution to total copepod assemblage (b) in the upper 50 m at the offshore Stn L20 (continuous line) and at the coastal Stn MC (dotted line).

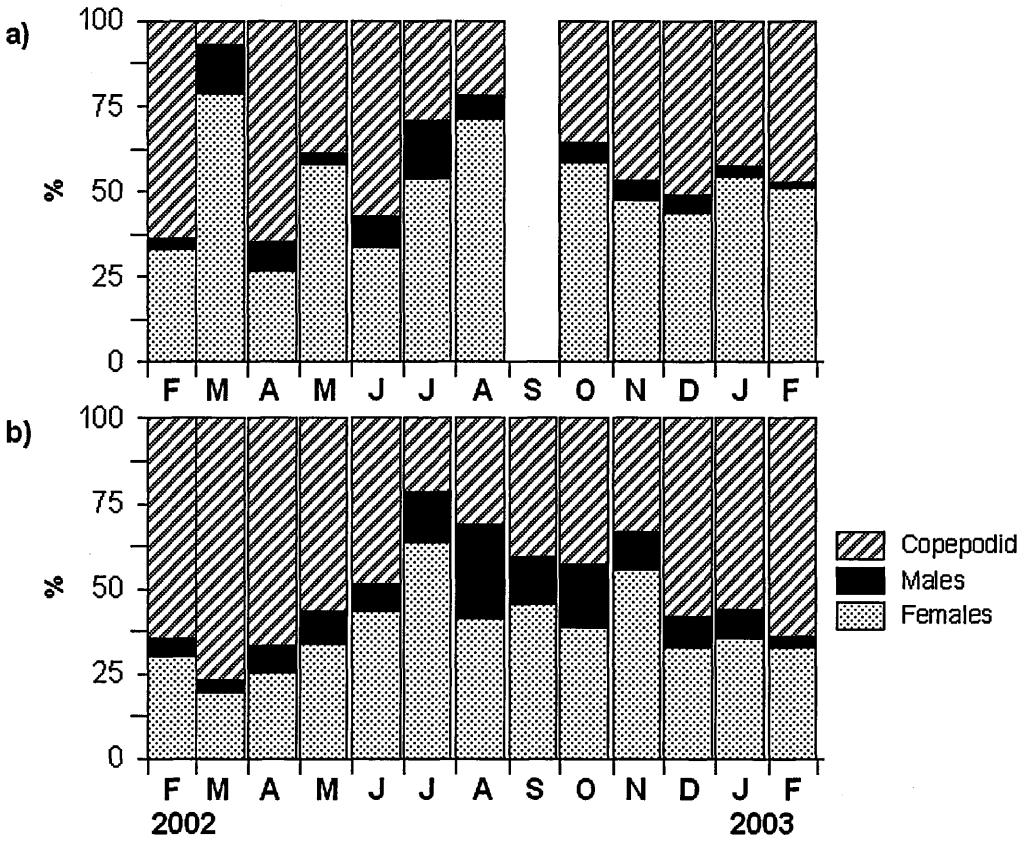


Figure 2.11. Population structure of the genus *Clausocalanus* in the upper 0-50 m at Stn L20 (a) and at Stn MC (b) in February 2002-February 2003. Sampling was missed in September at Stn L20.

In terms of population structure, copepodids contributed to total *Clausocalanus* abundance more in coastal than in offshore waters ($51.7 \pm 16.7\%$ and $42.1 \pm 17.2\%$, respectively)(Fig. 2.11). At Stn MC, their contribution followed seasonal variations and was highest in winter and spring. Males represented 10.6% (± 6.8) of adult abundances over the annual cycle at Stn MC and 6.9% (± 4.7) at Stn L20.

Three species dominated in both offshore and coastal waters: *C. furcatus*, *C. paululus* and *C. arcuicornis* (Table 2.5)(Fig. 2.12). In winter, the water column was dominated by *C. paululus* at both sites. *C. arcuicornis* increased its

Table 2.5. Mean specific contribution (% ±SD) of adult females in the 0-50 m at Stn L20 and Stn MC in the period February 2002-February 2003. September was not included because of missed sampling at Stn L20.

Species	Stn L20 (0-50 m)	Stn MC (F2002-F03)
C. paululus	29.3 (±28.5)	30.4 (±23.1)
C. pergens	3.0 (±3.6)	2.1 (±1.4)
C. furcatus	31.9 (±40.7)	31.5 (±32.4)
C. jobei	3.2 (±3.2)	2.0 (±2.5)
C. parapergens	2.6 (±2.2)	0.2 (±0.3)
C. arcuicornis	25.0 (±21.9)	27.7 (±20.0)
C. lividus	3.8 (±4.5)	4.4 (±5.7)
C. mastigophorus	1.2 (±1.4)	1.4 (±1.1)

contribution from winter to spring, with higher contribution in May at both sites. *C. furcatus* dominated in summer; it contributed earlier and with higher percentage offshore but it lasted longer until winter in coastal water. Among the less abundant species, *C. pergens* showed higher contribution at the offshore station. *C. lividus* contribution was, on the average, very similar at both sites and more important in spring and winter. *C. jobei* and *C. parapergens* highest relative contributions were observed in spring and autumn–winter at both stations. *C. mastigophorus* showed a higher contribution in late autumn–winter at both sites.

At both sites, *C. paululus* occurred in higher abundance from winter to early spring but it was more abundant offshore (Fig. 2.13a). *C. pergens* had a high peak in April only at the offshore station, occurring in very low abundance during the rest of the year and during the whole cycle at Stn MC (Fig. 2.13b). *C. furcatus* had overlapping seasonal patterns at both stations, but it was more abundant at Stn L20 where the highest summer peak occurred one month earlier than at Stn MC (Fig. 2.13c). *C. jobei* (Fig. 2.13d) and *C. arcuicornis* (Fig. 2.13f), despite

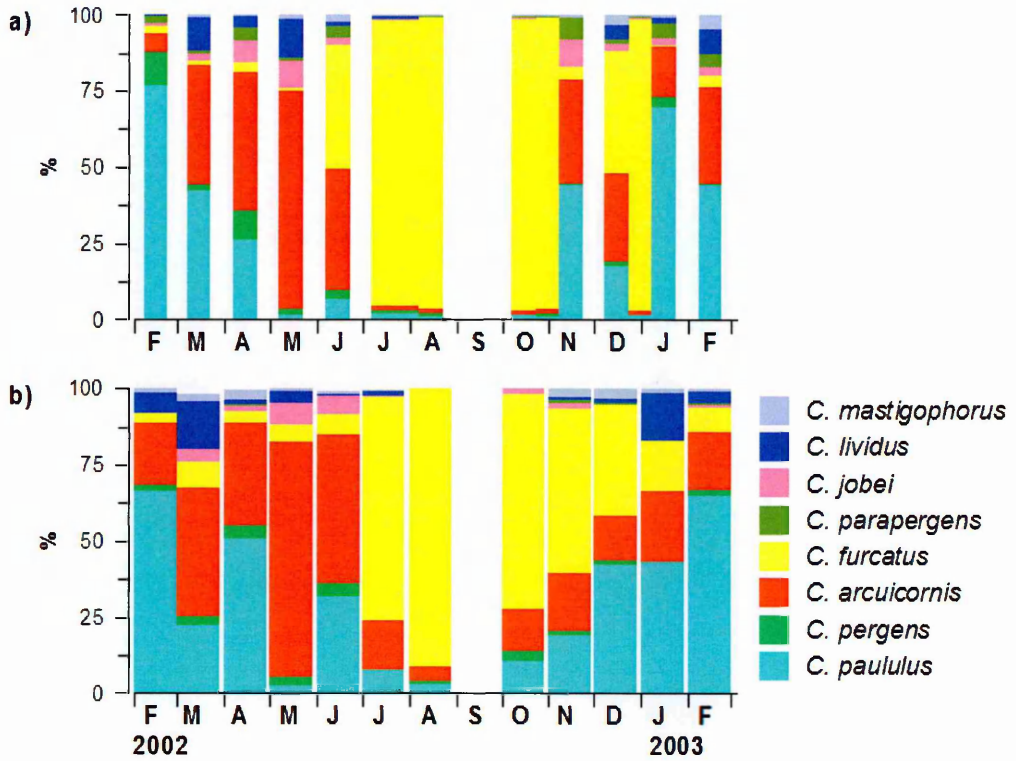


Figure 2.12. Relative contribution of *Clausocalanus* species (adult females) to total *Clausocalanus* females in the upper 0-50 m at the offshore Stn L20 (a) and at the coastal Stn MC (b) from February 2002 to February 2003. Sampling was missed in September at Stn L20.

the fact that the latter outnumbered the former, had very similar seasonal patterns and the spring peak was much higher offshore than at the coastal station. *C. parapergens* displayed three peaks in April, November and January at Stn L20 while at Stn MC its presence was negligible (Fig. 2.13e). *C. lividus* showed similar seasonal cycles at both stations; at Stn L20, the spring peak was higher and wider while the secondary peak occurred earlier but lower than at Stn MC (Fig. 2.13g). *C. mastigophorus* was similarly scarce at both stations, with slightly higher abundances offshore only on a few occasions (Fig. 2.13h).

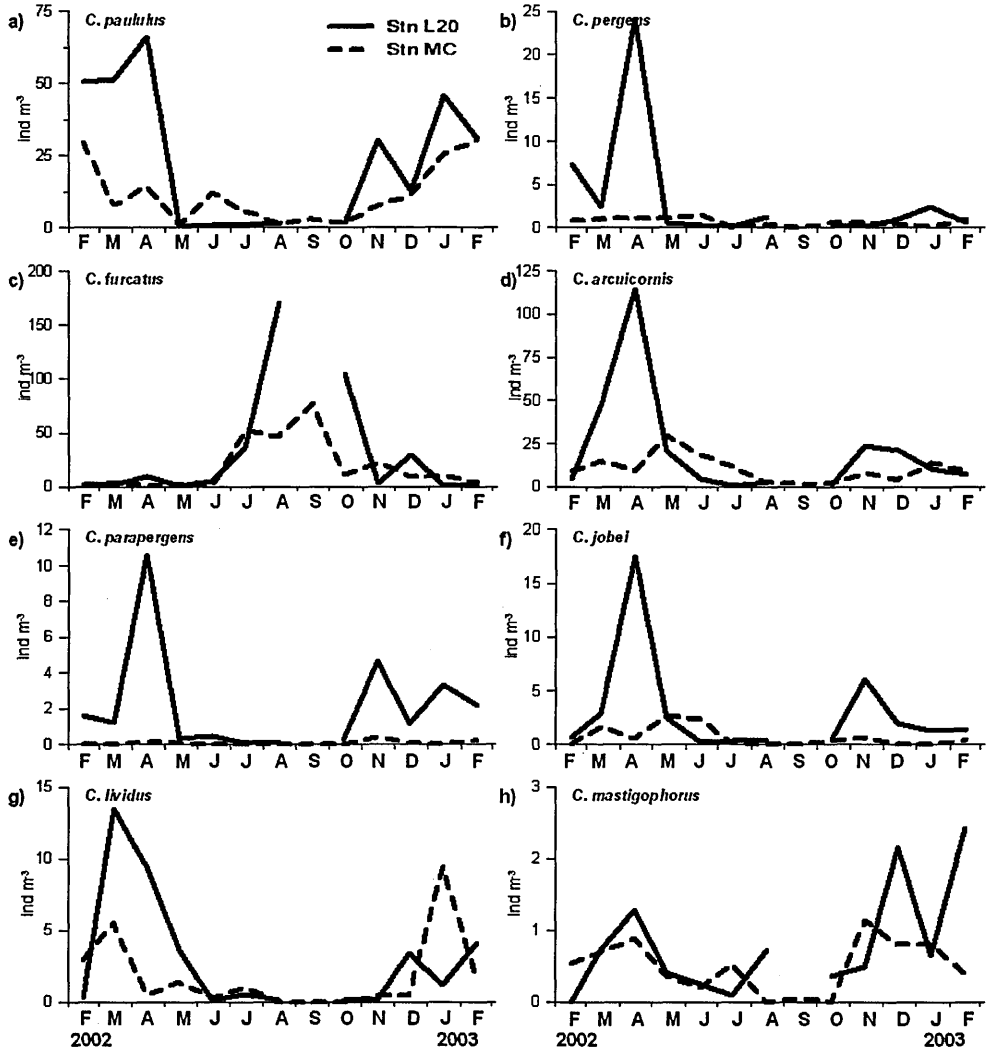


Figure 2.13. Seasonal distribution of *Clausocalanus* adult females in the 0-50 m layer at Stn L20 and Stn MC from February 2002 to February 2003.

2.3. Discussion

The present chapter provides a comprehensive picture of the vertical distribution of eight *Clausocalanus* species during an annual cycle in the upper 200 m water column at an offshore site (Stn L20) characterized by oligotrophic conditions. The patterns of species occurrence and abundance were complemented with data on sex ratio and reproductive index to provide some preliminary information on periods and depth layers where species reproductive activity occurs. The comparison of the same annual cycle in the upper 50 m layer at Stn L20 and at the coastal Stn MC, both in the Gulf of Naples, contributed to a better understanding of the offshore-oligotrophic/coastal-eutrophic generic and specific preferences.

In the year February 2002-February 2003, the two study sites were characterized by the same patterns of temperature and salinity. The former followed the typical cycle of temperate Mediterranean regions, while the latter showed an unusual rapid decrease in full summer due to intense rainfall, infrequent in this season in the area. The sites differed remarkably in the concentrations of autotrophic biomass, which confirmed the permanent oligotrophic conditions of Stn L20, where Chl *a* concentrations were up to thirteen times lower than those in coastal waters. At Stn L20 by late spring, a deep chlorophyll maximum (DCM) was established at the base of the thermocline (75-80 m), a typical feature in both the western and eastern Mediterranean basins (Longhurst, 2006), where it generally deepens through mid-summer (Estrada *et al.*, 1993). However, in the upper 50 m layer, Chl a concentrations were always low, as also recorded during another recent annual cycle (Di Capua and Mazzocchi, 2004), and did not show the notable spring and autumn peaks observed at the coastal station. At Stn MC, three different phytoplankton growth phases occur at the surface during the seasonal cycle: the winter and autumn blooms, which are very likely related to large

scale meteorological factors, and the late spring-summer blooms which are a local phenomenon driven by lateral advection of nutrients and biomass from coastward sites (Ribera d'Alcalà *et al.*, 2004).

At both stations, *Clausocalanus* represented an important numerical component of the copepod communities throughout the year. On an annual basis, the genus was more important offshore, both in terms of abundance (particularly for the high peak in April) and relative contribution to total copepod assemblages. The contribution was high at both sites in winter, the season of minimum zooplankton abundance and lowest autotrophic biomass (Ribera d'Alcalà *et al.*, 2004). Moreover in April, it seems that the genus did not take advantage over other copepods in coastal waters where a chlorophyll peak was recorded, while it increased its contribution offshore in very oligotrophic conditions. It seems therefore that even poor autotrophic biomass can be sufficient to sustain *Clausocalanus* populations, suggesting that their feeding habits are not strictly based on autotrophic resources. Most calanoids have actually been shown to be omnivorous, but *Clausocalanus* seems to be more adapted to low phytoplankton concentrations. Different aspects related to feeding in *Clausocalanus* have been investigated so far in a limited number of studies (see Table 1.2). Differently from all other small calanoids, *C. furcatus* moves continuously and rapidly, exploring small areas in close succession; it does not create a feeding current and captures the food particles after direct interception (Mazzocchi and Paffenhöfer, 1999). All species of the genus show similar swimming behaviour and, likely, the same mode of catching food. According to Uttieri *et al.* (in press), *C. furcatus* apparently collects particle food almost like a passive sieve since only perceives those prey located in the close vicinity of its rostrum. Nevertheless, these authors observed that this species handles and occasional rejects the intercepted cells what seem to indicate

a selection likely by the second antennae and mouthparts due to palatable versus unpalatable prey. Numerous apical-pore setae that *C. furcatus* bears on its second antennae (Uttieri, 2006) indicates that this copepod perceives, rather than mechanical, chemical compounds of phytoplankton cells during the movement of its cephalic appendages (Uttieri *et al.*, in press). As a consequence, *Clausocalanus* could feed on a wide range of food resources and, owing to its swimming behaviour, be adapted to exploit small patches of food items, as recently hypothesized by Uttieri (2006). The presence of oil sacs in *Clausocalanus* females observed at Stn L20 in winter might represent stored reserves and contribute to overcome periods of very scarce food resources.

On an annual basis, the structure of *Clausocalanus* assemblages was striking similar at Stn L20 and Stn MC; all species contributed in a similar way at the two sites and only the less abundant species (*C. pergens*, *C. jobei*, *C. parapergens*, and *C. mastigophorus*) ranked in a slightly different order. This suggests that over an annual cycle on a local scale (the Gulf of Naples), no spatial partitioning could be discerned among *Clausocalanus* species between offshore / coastal conditions, at least in the upper 50 m layer and in the year under consideration. Adults were relatively more abundant offshore, while copepodids were relatively more abundant in coastal waters. This difference could result from two alternative or concurrent reasons. In coastal areas, a more intense reproductive activity (more juveniles) is expected to occur because of more food availability, but also adult mortality does likely increase because of selective predation (adults larger than juveniles) by more abundant pelagic carnivores. Neither of the two hypotheses can be tested with zooplankton data available so far in the Gulf of Naples, but unpublished data have shown that predation by chaetognaths on copepods at Stn MC can be considered negligible on *Clausocalanus* populations (P. Simonelli and M.G. Maz-

zocchi, personal communication). The annual distribution of *C. arcuicornis* and *C. paululus* copepodid stages was observed by Shmeleva and Kovalev (1974) in the Adriatic Sea. Copepodid stages represented 55-90% of *C. arcuicornis* population, and 60-80% of *C. paululus* population.

All *Clausocalanus* species showed strong seasonal signals in their occurrence, with very similar patterns at Stn L20 and Stn MC, as should be expected. Only *C. pergens* and *C. parapergens* did not show any seasonal cycle at Stn MC in the study year because of their negligible numbers. Mazzocchi and Ribera d'Alcala (1995) observed recurrent patterns in the seasonal cycles of *C. paululus*, *C. pergens*, *C. arcuicornis*, and *C. furcatus*, with a clear seasonal succession of their peaks from winter to autumn, a succession that can be discerned also in the datasets analysed in the present study and seems to be therefore characteristic of these populations in the area. *C. pergens*, which was among the most abundant species in the first years of the time-series at Stn MC (Mazzocchi and Ribera d'Alcala, 1995), was among the less abundant species in the annual cycle here analysed at the Stn MC where its seasonal pattern could not be discerned. Apparently, this species has notably reduced its occurrence at Stn MC as already noticed by Mazzocchi and Ribera d'Alcala (1995).

The medium sized *C. arcuicornis*, *C. parapergens*, and *C. jobei* had the same period of highest abundance (spring), but *C. arcuicornis* was by far the dominant species. Studies conducted on the horizontal distribution of copepods in the Mediterranean Sea noted different abundances of these *Clausocalanus* species depending on the study areas Hure and Scotto di Carlo (1970); Regner (1976); Pancucci-Papadopoulou *et al.* (1992); Fragopoulu *et al.* (2001). This suggests that the three species probably have different horizontal distributions at the sub basin scale. *C. mastigophorus* and *C. lividus*, the largest *Clausocalanus* species present

in the Mediterranean Sea, were very scarce in the Gulf of Naples, and reached peak abundances in spring in the upper layer. Their occurrence in the upper layer during their peak of abundance might prevent egg loss by sinking and ensure egg hatching in the upper photic zone where nauplii can find more food.

In the North Atlantic, Williams and Wallace (1975) reported differences in the seasonal cycles and in the relative abundance of some *Clausocalanus* species. The total abundance of all species of *Clausocalanus* was mostly represented by *C. pergens*, *C. lividus*, and *C. arcuicornis* (80.4%). In the Gulf of Naples, *C. lividus* never occurred in high numbers, whereas *C. paululus* was among the most abundant species. Williams and Wallace (1975) did not record *C. paululus* in the North Atlantic (north of 40°N), and this could be due two different causes: the mesh size used for the CPR was too large (300 μm) to catch the smallest *Clausocalanus* species, or *C. paululus* latitudinal distribution was more restricted than the surveyed region. Frost and Fleminger (1968) described the distribution of *C. paululus* as apparently subtropical, but no data were available at that time for the North Atlantic, where its presence has been reported only recently by Schnack-Schiel and Mizdalski (2002). *C. mastigophorus* was the rarest species of the genus (Williams and Wallace, 1975) while, Fragopoulu *et al.* (2001) found that *C. mastigophorus* was the fifth most abundant species of the genus and *C. lividus* the rarest in the Eastern Mediterranean. These observations suggest different latitudinal gradients of abundance for the two species, although this does not appear from the qualitative maps of distribution drawn by Frost and Fleminger (1968). The seasonal cycles of *Clausocalanus* species observed by Williams and Wallace (1975) were very similar to these observed at Stn L20. *C. furcatus* was recorded only in the western Atlantic around 43°N (the southern surveyed area) occurring in low abundances and peaking in September and November (Williams and Wallace,

1975). *C. furcatus*, the most abundant *Clausocalanus* species at Stn L20, was concentrated in summer-autumn, when phytoplankton concentrations are low even in coastal waters (Mazzocchi and Ribera d'Alcala, 1995; Ribera d'Alcalà *et al.*, 2004). It was important also in extremely oligotrophic waters such as the open Eastern Mediterranean (Siokou-Frangou *et al.*, 1997), suggesting that the metabolic needs of this species are satisfied even at low levels of autotrophic biomass, as shown by feeding experiments in the laboratory (Mazzocchi and Paffenhöfer, 1998) and in the field (Paffenhöfer *et al.*, 2006).

Adult *Clausocalanus* species showed some differences in their vertical distribution at Stn L20. In April, when the thermocline was not yet completely stabilized and the DCM occurred at 60 m, six *Clausocalanus* species were concentrated in the upper 0-20 m layer and only *C. paululus* and *C. parapergens* were localized deeper (20-50 m). From July to October, *C. furcatus* dominated the upper narrow mixed layer where water temperature exceeds 20°C. Meanwhile the rest of the *Clausocalanus* species had the core of their populations at the DCM. In autumn, when the mixed layer deepened and also the DCM occurred deeper, the *Clausocalanus* populations were found in the upper layer. In winter, in a thoroughly mixed water column, the distribution of the species did not show preferential depths. It seems that seasonal changes in the structure of the water column create environmental conditions sufficiently diversified (in physical properties) to permit species coexistence.

Over a larger vertical scale in Tyrrhenian waters (0-3000 m), *Clausocalanus* species showed different depth ranges of occurrence; *C. furcatus* was more restricted to the upper layer (0-100 m) and *C. lividus* (0-3000 m) and *C. mastigophorus* (0-2000 m) the most widely distributed (Scotto di Carlo *et al.*, 1984). *C. furcatus* is a warm-water species and its distribution is typically superficial,

above the thermocline both in the Mediterranean (Fragopoulu and Lykakis, 1990) and in the Sargasso Sea (Paffenhöfer and Mazzocchi, 2003). The vertical distribution of *C. paululus* and *C. arcuicornis* observed at Stn L20 was similar to that observed by Shmeleva and Kovalev (1974) in the Adriatic Sea. Both Fragopoulu and Lykakis (1990) in the Patraikos Gulf (Greece) and Paffenhöfer and Mazzocchi (2003) in the Sargasso Sea observed different responses to the development of the thermocline between *C. furcatus* and *C. pergens*. *C. furcatus* remained above the thermocline while *C. pergens* remained below.

The vertical distribution of the *Clausocalanus* population structure observed at the offshore station during the annual cycle did not reveal any apparent ontogenic migration in the epipelagic zone. Copepodids were always more concentrated in the upper layer, which they seemed to avoid only in June-July. However, some weak ontogenetic vertical migrations could have been masked by the missed identification of copepodites at the species level. Controversial evidence is reported in the literature regarding diel vertical migration in *Clausocalanus* genus and species (e.g. Timonin, 1977; Checkley *et al.*, 1992; Zunini Sertorio and Licandro, 1994; Vega-Pérez *et al.*, 1999; Lo *et al.*, 2004). Not all species seem to undergo vertical migration, and in those in which it has been observed, migration patterns are not strong.

All *Clausocalanus* species were always present in the water column at Stn L20 all year round (the only exception was the absence of *C. mastigophorus* in February 2002), indicating continuous reproduction sufficient to sustain the persistence of populations even at low numbers.

Gender distribution was strongly skewed to females in all *Clausocalanus* species, but in *C. lividus* and *C. mastigophorus*, predominance of males was observed in some occasions. The low abundance of *Clausocalanus* males observed at Stn L20 is

in accordance with the observations of Gaudy (1972, *C. furcatus* and *C. arcuicornis*) for the Gulf of Marseille, of Shmeleva and Kovalev (1974, *C. arcuicornis* and *C. paululus*) in the Adriatic Sea, and Kouwenberg (1993, genus *Clausocalanus*) in the north-western Mediterranean. Predominance of females over males occurs in those copepod species that have seminal receptacles where sperm can be stored for various successive spawnings (Ohtsuka and Huys, 2001). In these species, males have reduced or lost feeding abilities, high energetic expenditure because of fast swimming behavior (enhancing encounter rates with mates but also predators) and therefore their life is much shorter than that of females (Kiørboe, 2006). Highest sex ratios were observed in the broadcast spawners *C. lividus* and *C. mastigophorus* and could be biased by the very low adult abundance, or be a specific reproductive trait of these spawning mode. Broadcast spawners could need higher percentage of fertilized females, with respect to the sac-spawning congeneric species to ensure their population maintenance since free eggs have higher mortality. In sac-spawner species, sex ratio, reproductive index and adult abundance were correlated in most of the species. In any case, and although in low numbers, the presence of *Clausocalanus* males was sufficient to sustain the populations throughout the year.

Clausocalanus reproductive index estimated on females carrying at least one spermatophore or egg sac remains differed among species. *C. arcuicornis* and *C. paululus* showed by far the highest RI, while those of the remaining species were tenfold times lower. The RI of *C. furcatus* at Stn L20 was similar to those reported by Webber and Roff (1995) for the Caribbean Sea (mean RI: 0.8, range 0-9%), low in relation to other egg-carrying calanoids. The authors attributed the low observed RI to the easy dislodging of the egg mass in this species. At Stn L20, even the small *C. paululus*, which showed very few females with sac

remains had much higher percentage of females with attached spermatophore(s) than *C. furcatus*. But when looking at spermatophore size (two different spermatophore sizes are present in the genus; Frost and Fleminger, 1968) it can be noted that *C. paululus*, *C. arcuicornis* and *C. jobei* have large spermatophores while the other species have short ones. Large spermatophores would take more time in pouring their contents to the seminal receptacle enhancing the probability of observing females with attached spermatophores. In fact, different pouring phases were observed in large spermatophores while most of the observed short spermatophores were empty. Multiple spermatophore attachment was frequently observed in most of the *Clausocalanus* species occurring at Stn L20 and also in *C. paululus* in different Mediterranean environments (M.G. Mazzocchi, personal communication). *Clausocalanus* present seminal receptacles and males are few and have reduced feeding appendages (Frost and Fleminger, 1968), features suggesting that remating is not necessary in *Clausocalanus* species. On the contrary, multiple spermatophores observed at high frequency indicate undoubtedly multiple mating events.

Peaks of RI in *Clausocalanus* coincided with peaks of sex ratio, both seasonally and vertically, especially during adult peak abundances. Gaudy (1972), based on weekly or biweekly sampling in the Gulf of Marseille, assessed the number of generations of *C. arcuicornis* and *C. furcatus* from adult abundances. In the Gulf of Marseille, *C. arcuicornis* and *C. furcatus* had five generations: the first in January-March, the second in April-May (*C. arcuicornis* in April, *C. furcatus* in May), the third in July-August (sometimes until September), the fourth in late September-October (*C. furcatus* in September, *C. arcuicornis* in October), and the fifth in November-December. On the other hand, Shmeleva and Kovalev (1974) observed only three clear generations in *C. arcuicornis* in the Adriatic Sea

(in winter, spring and summer), but five generations of variable duration over the year in *C. paululus*, the longest one from November to mid-March. The monthly sampling frequency of the present study and the lack of copepodid identification at the species level did not allow the assessment of the number of generations of *Clausocalanus* species in the open Gulf of Naples. However, the distribution of sex ratio and reproductive index show clearly that *C. paululus* and *C. arcuicornis* reproduce throughout the annual cycle as observed in the Adriatic Sea (Shmeleva and Kovalev, 1974). In all the other species, higher reproductive activity can be noticed in at least 3-5 periods along the annual cycle.

In summary, the small species *C. paululus* was more abundant and its peak in abundance occurred earlier than that of the similar in size *C. pergens*. The medium sized species *C. arcuicornis*, *C. parapergens*, and *C. jobei* had similar seasonal cycles, but the peak in abundance of *C. parapergens* occurred deeper in the water column. *C. arcuicornis* and *C. jobei* were concentrated in the upper layer during their peaks, with the former outnumbering the latter. *C. furcatus* differed from the other species by showing a narrow and quite restricted period and depth of occurrence. The large *C. mastigophorus* and *C. lividus* overlapped in seasonal and vertical distribution, but both species occurred in low abundances. In conclusion, the detailed analysis of the temporal and vertical occurrence of *Clausocalanus* species in relation to certain environmental parameters allowed me to depict a coherent picture of their distribution in the Gulf of Naples and make hypotheses regarding the differentiation of their niches, which will be further developed in Chapter 5.

CHAPTER 3

Spring distribution in two different trophic regimes

3.1. Oligotrophic conditions in the Ionian Sea (Eastern Mediterranean)

The Eastern basin (EMED) is the most oligotrophic region in the Mediterranean Sea (Azov, 1991). Its complex mesoscale physical dynamics plays a crucial role in the circulation of the entire Mediterranean (Robinson *et al.*, 1991; POEM-group, 1992). In the last two decades, the region has undergone relevant changes in its deep and intermediate circulation as a transient effect of climate forcing named EMT (Eastern Mediterranean Transient)(Roether *et al.*, 1996; Malanotte-Rizzoli *et al.*, 1999a). The notable amount of information now available on the evolution of the EMT and its effects on the thermohaline circulation of the EMED is widely discussed in Manca (2000). The Ionian Sea in the EMED represents an important area for the dynamics of water masses originating in the western and eastern basins. It is an oligotrophic sea where autotrophic biomass is low (1-8 μg C L^{-1}) and mainly constituted by picoplankton (Rabitti *et al.*, 1994; Casotti *et al.*, 2003). The Ionian Sea has been affected by EMT in deep layers below 1000 m and its follow up has caused a dramatic reversal of its upper circulation which has become cyclonic since March 1998 (Manca, 2000). In addition, the EMT has determined

an uprise of the nutricline in the northern and eastern Ionian Sea, which carried nutrients close to the euphotic zone (Klein *et al.*, 1999), and showed some effects also on epipelagic mesozooplankton communities (Mazzocchi *et al.*, 2003).

In the spring of 2002, a survey was conducted in the open Ionian Sea in the frame of the Italian programme SINAPSI. The programme was focused on biogeochemical and physical processes in key marine areas for monitoring the ecosystem variations at the seasonal, interannual and decadal scales in the Mediterranean (Pinardi, 2000).

The hydrology of the Ionian Sea during cruise SINAPSI-4 was described by Manca *et al.* (2006), who analysed CTD casts in the whole water column but focused mainly on the intermediate and deep circulation. Through the shallow Sicily Strait, the fresher water of Atlantic origin (Modified Atlantic Water, MAW) flowed in the surface layer (<200 m) of the Ionian Sea from west to east, whereas a more saline and warmer core water intruded into the Ionian from the eastern side and flowed predominantly northwards along the Greek coast. This latter water mass mixed with the MAW and became the Ionian Surface Water (ISW). A sharp temperature and salinity front at 36°N separated the ISW in the north from the MAW to the south, where it flowed mainly eastwards with an anticylonic motion. The ISW was laterally bounded on its western side by a water mass characterised by low temperature and high salinity. This was probably caused by the cyclonic dynamics in the area, so that cold and salty waters were lifted from the intermediate layer close to the surface. The ISW flowed northward and extended into the Adriatic Sea, having a high salinity core because of the cyclonic circulation in this region.

In the western Ionian, the salinity and temperature values of the MAW ranged from 37.6 to 38.6 psu and from 15 to 17°C, respectively (Manca *et al.*, 2006). It

occupied the 0-150 m layer at Stns C09, C08, C07 and could be still recognized at Stn C06, whereas a small quantity of MAW appeared at Stns T02 and G99. The MAW, as it moved eastward, mixed with the more saline ISW, which could be well identified in the eastern and northern Ionian by salinities greater than 38.70 psu.

In SINAPSI-4, mesozooplankton was preliminary analysed to investigate inter-annual variations in its abundance and trophic structure in the epipelagic domain throughout the onset, evolution and decline of the EMT (Malanotte-Rizzoli *et al.*, 1999b).

In this thesis, the *Clausocalanus* assemblages are examined in detail to depict their composition and spatial distribution in open oligotrophic waters.

3.1.1. Materials and methods

SINAPSI-4 cruise was carried out in the Ionian Sea from 27 March to 10 April 2002 onboard the R/V *Urania*. Mesozooplankton samples were collected at 16 stations spread throughout the basin (Fig. 3.1; Table 3.1). At each station, a vertical tow was performed with a Nansen net (1 m^2 mouth area, 200 μm mesh size) in the upper 100 m of the water column. Samples were fixed onboard immediately after collection and preserved in a buffered formaldehyde-seawater solution (4% final concentration).

In the laboratory, zooplankton were identified and counted under a stereoscope using a Bogorov chamber. At least $1/10^{th}$ of the entire sample was analysed taking repeated aliquots with a large mouth graduated syringe after accurate mixing (modified Stempel pipette method). *Clausocalanus* females were identified at the species level according to Frost and Fleminger (1968), while males and juveniles where identified at the genus level (counts performed by M.G. Mazzocchi, SZN,

Laboratory of Bilogical Oceanography).

In order to compare *Clausocalanus* species composition with water characteristics, the environmental data (temperature, salinity and fluorescence) of the upper 100 m recorded by CTD (seabird SBE 911-plus CTD rosette system)(data provided by Dr. M. Ribera d'Alcalà) were depth averaged and mapped using kriging (a geostatistic analysis) and statistically analyzed with SPSS 12 statistical package. Statistical analysis was performed on standardized data (Z values) of the environmental parameters in order to minimize scale effects (Legendre and Legendre, 1998). Hierarchical clustering (based on the average linkage classification) and nonmetric multidimensional scaling (MDS) were performed. The combination of clustering and ordination analysis allows evaluation of the adequacy and mutual consistency of both representations (Legendre and Legendre, 1998).

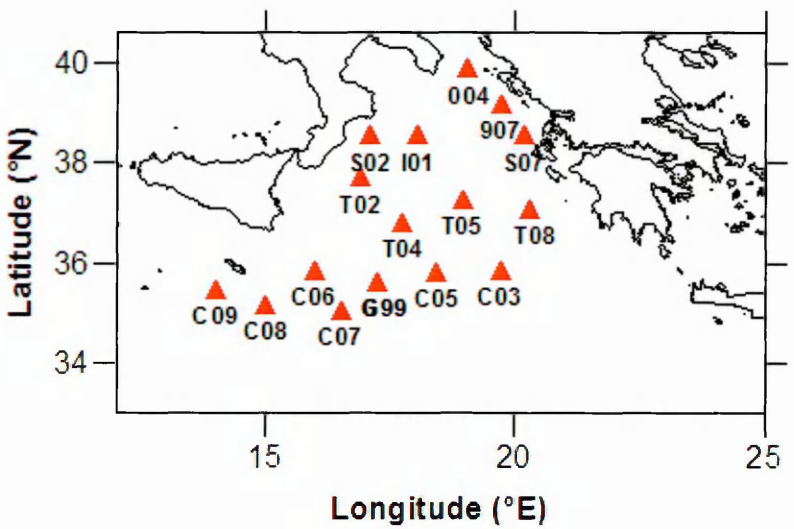


Figure 3.1. Map of the Ionian Sea with stations sampled for mesozooplankton collection during SINAPSI-4 cruise in spring 2002.

Table 3.1. Zooplankton station data for the SINAPSI-4 cruise in the Ionian Sea. Collection time is reported in Coordinated Universal Time (UTC), a high-precision atomic time standard.

STN	DATE	TIME	COORDINATES		SONIC
	(dd/mm/yy)	(UTC)	Lat. (°N)	Long. (°E)	DEPTH (m)
C09	30/03/02	7:51	35°24.97	13°59.98	579
C08	30/03/02	15:15	35°08.00	14°59.98	556
C07	31/03/02	0:45	34°59.98	16°29.92	2296
C06	31/03/02	16:46	35°48.00	15°57.95	3584
1G99	01/04/02	13:02	35°34.98	17°15.00	4012
SG99	01/04/02	18:15	35°34.98	17°15.00	4012
C05	02/04/02	1:58	35°46.98	18°26.05	4053
T04	02/04/02	13:04	36°45.13	17°45.17	3420
T02	02/04/02	23:10	37°40.00	16°54.02	2350
S02	03/04/02	8:32	38°30.03	17°04.98	1565
I01	04/04/02	8:00	38°29.76	18°03.18	2377
S07	05/04/02	16:49	38°29.96	20°09.94	2758
T08	06/04/02	10:47	37°01.25	20°16.89	3201
C03	06/04/02	19:42	35°47.96	19°41.98	3172
T05	07/04/02	7:44	37°13.32	18°56.23	3327
907	07/04/02	20:40	39°07.06	19°41.96	1503
004	08/04/02	6:09	39°50.02	19°09.78	937

3.1.2. Results

Environmental parameters

The vertical profiles of environmental parameters in the upper 200 m layer, showed that the water column was mixed at all stations, with deep chlorophyll maxima (DCM) present between 50-100 m depth at most of the sites (Fig. 3.2). Fluorescence was higher at Stn S02 throughout the upper 50 m of the water column, and indicated the presence of the so called "Calabrian bloom" (D'Ortenzio, 2003).

Maps based on CTD data integrated in the upper 100 m revealed northward

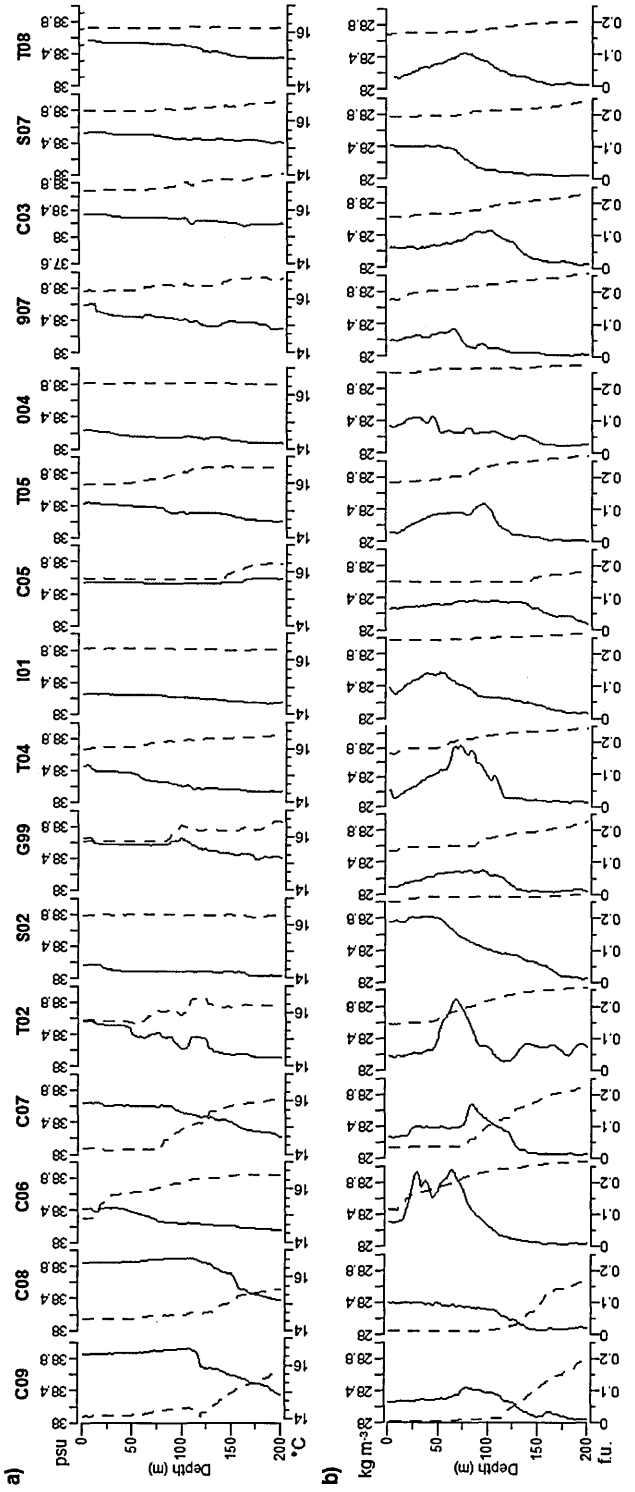


Figure 3.2. Vertical profiles of the environmental parameters recorded by CTD in the upper 200 m at stations surveyed for mesozooplankton in the Ionian Sea in spring 2002. (a) temperature (°C; simple line) and salinity (psu; discontinuous line); (b) fluorescence (f.u.; simple line) and density (kg m^{-3}; discontinuous line).

gradients of temperature (decreasing 2°C) and salinity (increasing 0.8 psu), and an eastward gradient in fluorescence (decreasing 0.2 f.u.)(Fig. 3.3).

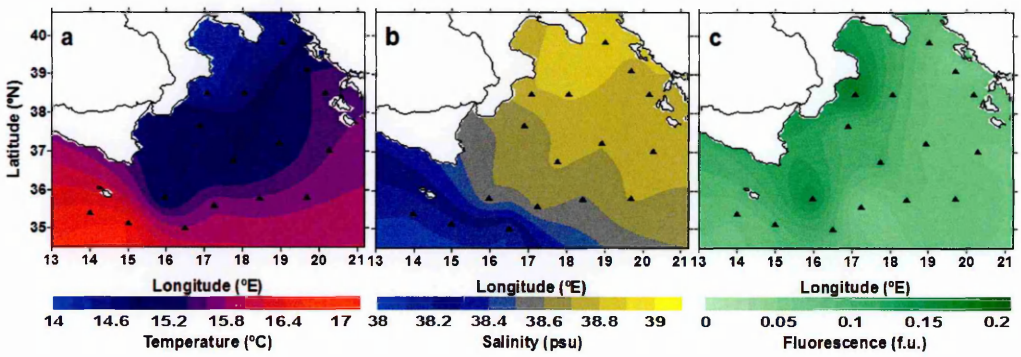


Figure 3.3. Distribution of environmental parameters in the Ionian Sea in spring 2002: (a) temperature (°C), (b) salinity (psu) and (c) fluorescence (f.u.) integrated in the 100-0 m.

Two major clusters of stations grouped by similar environmental characteristics were identified (Fig. 3.4):

- Cluster I (Stns C09, C08, C07) was influenced by warmer (15.81-16.60 °C), lower salinity (38.10-38.16 psu) and lower fluorescence (0.08-0.10 f.u.) water mass, which corresponded to the Modified Atlantic Water (MAW) that flowed into the Ionian Sea from the Sicily Strait;

- Cluster II included all the remaining stations. Three subgroups could be identified in Cluster II:

  - Cluster IIa was formed by two stations differing in their environmental parameters: Stn C06 was occupied by ISW, which had features resembling the MAW (15.05°C, 38.58 psu) but higher fluorescence (0.15 f.u.) and Stn S02 which was occupied by ISW (14.31°C; 38.79 psu) and presented the highest values of fluorescence of the area (0.17 f.u.);

- Cluster IIb (Stns 004, I01, T04) was formed by stations located in the northern Ionian Sea, characterized by presenting the coldest water mass (14.51-14.98°C), the core of salinity maximum (38.71-38.81 psu), and higher fluorescence (0.08-0.11 f.u.);
- Cluster IIc (Stns G99, C05, T08, C03, S07, T05, 907, T02) was influenced by colder (15.14-15.75°C), saltier (38.59-38.80 psu) and oligotrophic (0.06-0.08 f.u.) Ionian Surface Water (ISW).

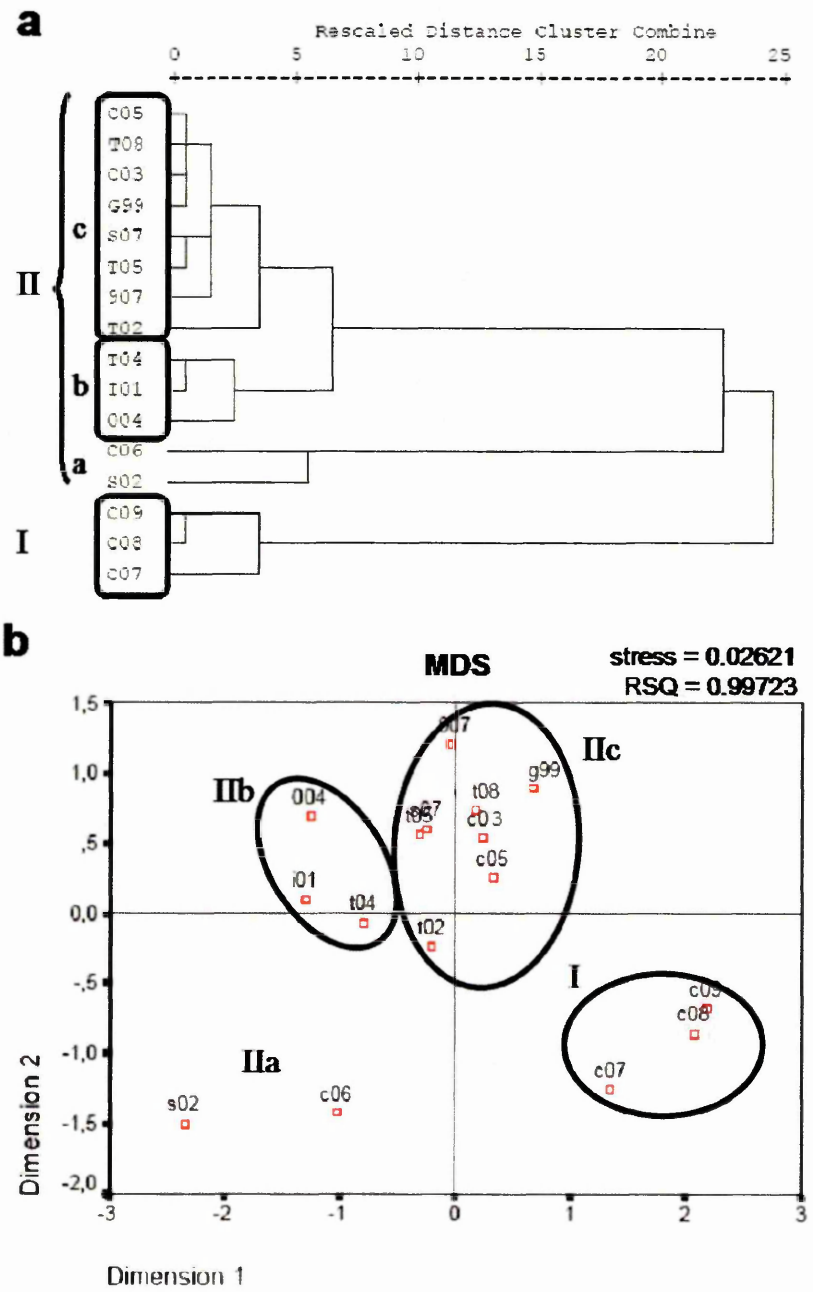


Figure 3.4. Groups of stations with similar environmental characteristics revealed by statistical analysis of integrated values of the environmental parameters (100-0 m; temperature, salinity and fluorescence) recorded by CTD in the Ionian Sea in spring 2002: a) dendrogram of the cluster analysis; b) non-metric multidimensional scaling plot (stress=0.02621 and squared correlation=0.99723). Station groups are indicated in circles and Roman numberals.

Clausocalanus

The abundance of *Clausocalanus* (adults and juveniles) in the upper 100 m water column ranged from 46.4 to 182.2 ind. m^{-3} (average 101.2 ± 41.9 ind. m^{-3})(Fig. 3.5a), accounting for 12.6-55.1% of the total copepod numbers (average $33.5 \pm 14.2\%$)(Fig. 3.5b).

Copepodids made up 45.8% (± 19.1) of total *Clausocalanus* abundance, representing up to 95.1% at Stn S02 (Fig. 3.5c). Females accounted for $94.4 \pm 2.9\%$ of adult *Clausocalanus*, while the male contribution was very low ranging from 2.1% to 11.6% (average $5.6 \pm 2.9\%$) (Fig. 3.5c).

All eight species recorded in the Mediterranean are found in the Ionian Sea (Fig. 3.5d). Only three species (*C. paululus*, *C. furcatus* and *C. arcuicornis*) were present at all stations, while the other five species had scattered occurrence, with *C. mastigophorus* and *C. pergens* being the least frequent (88.2% and 70.6% of the visited stations, respectively). *C. paululus* was by far the dominant species in the area, accounting for 38.8-82.0% of *Clausocalanus* female abundance in 82.4% of the stations. *C. arcuicornis* and *C. lividus* followed in a rank order of abundance, *C. arcuicornis* representing 25.9-28.7% at Stn C06 and C07 while *C. lividus* represented up to 56.3% at Stn S02 (Table 3.2).

At the species level, *Clausocalanus* females showed specific patterns of horizontal distribution (Fig. 3.7): *C. paululus* showed a gradient toward the eastern part of the basin, where it dominated (maximum at Stn T05, 51.4 ind. m^{-3})(Fig. 3.7a). *C. pergens* was absent at five stations (C05, T02, S02, S07 and T08) and showed its peak abundance at Stns C09 and 004 (3 and 6.4 ind. m^{-3}, respectively; Fig. 3.7b). *C. furcatus* was present at all stations and showed its peak abundance at Stns C07 and T05 (8.4 and 7.8 ind. m^{-3}; Fig. 3.7c). *C. jobei*, *C. parapergens* and *C. arcuicornis* had similar horizontal distribution (Fig. 3.7d–f) but the latter

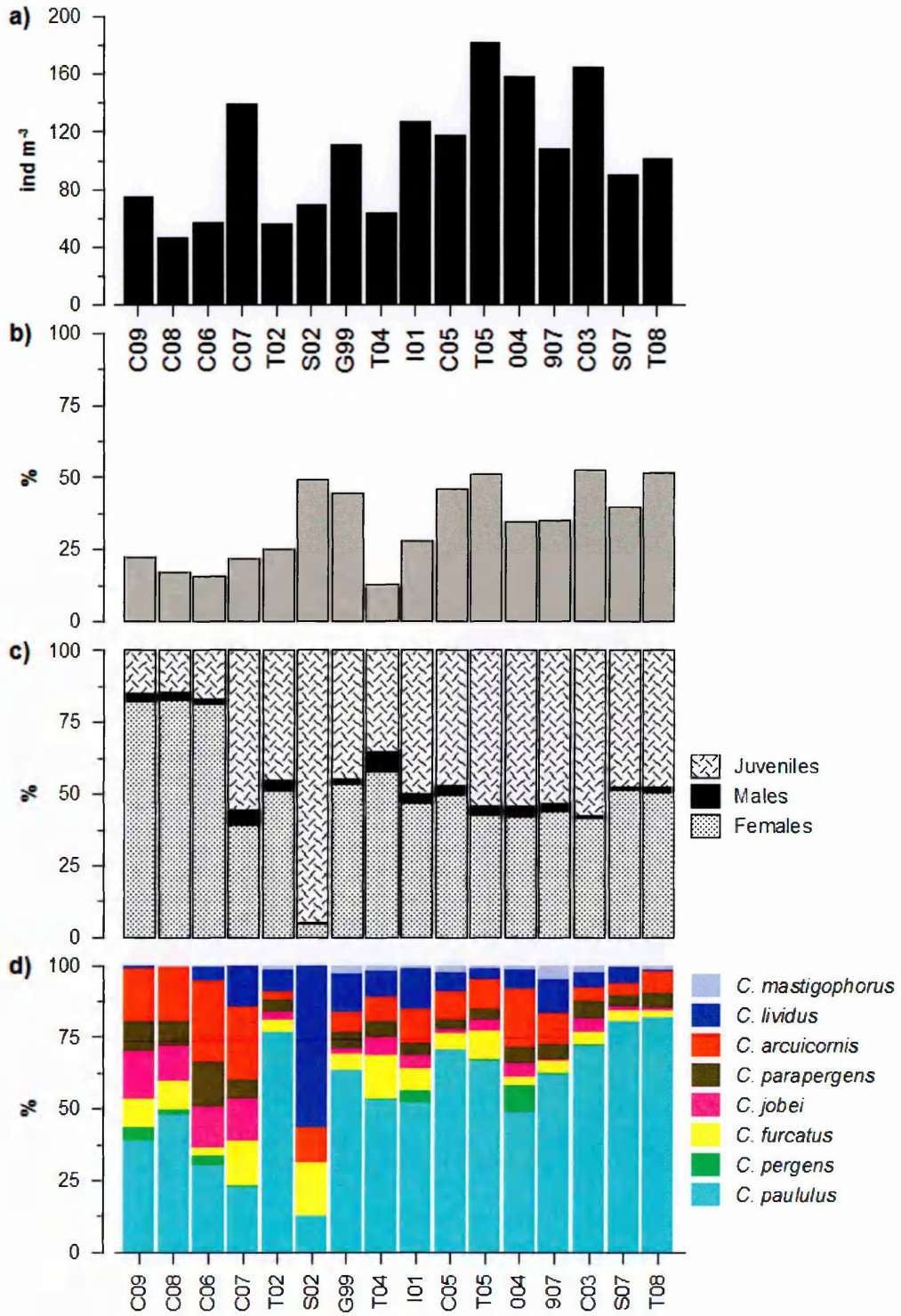


Figure 3.5. Ionian Sea. Total *Clausocalanus* abundance (a); percentage contribution of total *Clausocalanus* of total copepod abundance (b); *Clausocalanus* population structure (c); percentage contribution of females *Clausocalanus* species of total genus female abundance (d).

Table 3.2. Relative percentage contribution of *Clausocalanus* species (adult females) of total female abundance in the Ionian Sea in spring 2002.

Species	Mean	*sd*	min	max
C. paululus	55.4	20.3	12.5	82.0
C. pergens	1.5	2.6	0.0	9.6
C. furcatus	7.5	5.1	2.0	18.8
C. jobei	5.4	5.5	0.0	16.8
C. parapergens	5.9	3.7	0.0	15.7
C. arcuicornis	12.3	7.6	2.8	28.7
C. lividus	10.2	12.9	0.0	56.3
C. mastigophorus	1.6	1.4	0.0	4.7

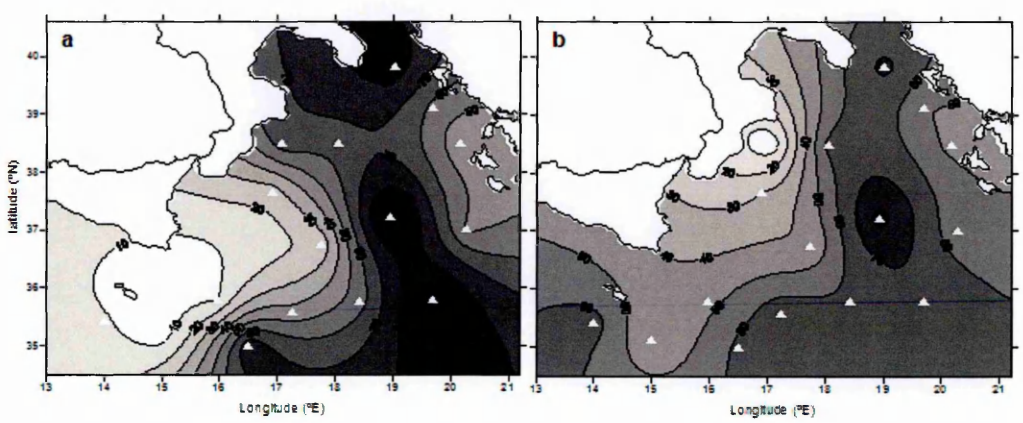


Figure 3.6. Horizontal distribution of *Clausocalanus* in the upper 100 m in the Ionian Sea: juveniles (a) and adults (b). Note that each isocline represents 10 ind. m^{-3}, white being indicative of low abundance and black of high abundance.

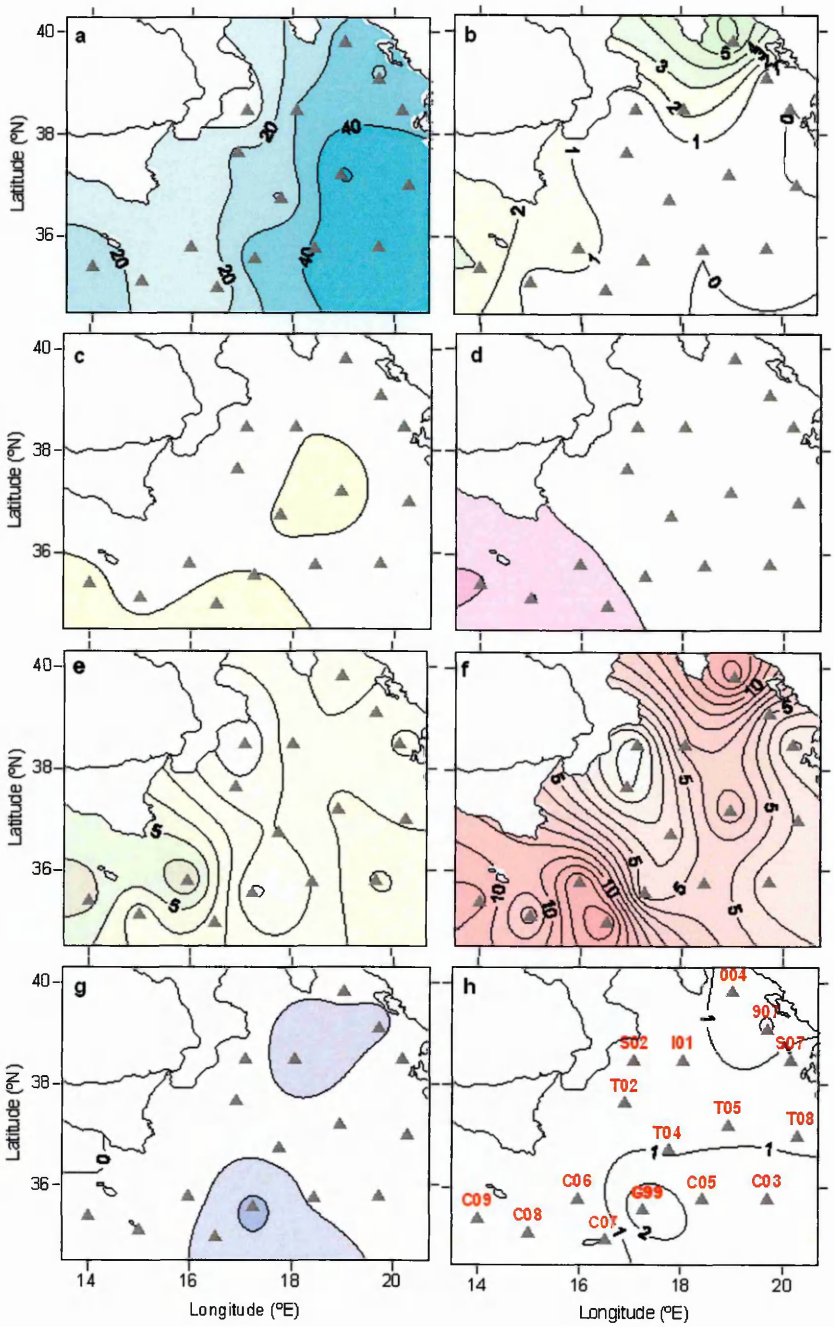


Figure 3.7. Horizontal distribution of *Clausocalanus* species (adult females) in the upper 100 m in the Ionian Sea in spring 2002. (a) *C. paululus*; (b) *C. pergens*; (c) *C. furcatus*; (d) *C. jobei*; (e) *C. parapergens*; (f) *C. arcuicornis*; (g) *C. lividus*; (h) *C. mastigophorus*. Note that each isoline represents 1 ind. m^{-3} except for (a), where it represents 10 ind. m^{-3}.

species was the most abundant of the three. *C. jobei* and *C. parapergens* peaked at Stn C09 and Stn C6, respectively (*C. jobei* 10.4 ind. m^{-3} and *C. parapergens* 7.2 ind. m^{-3}). Both *C. jobei* and *C. parapergens* were absent at Stn S02. *C. arcuicornis* was abundant at the south-western stations and at Stn 004 (14.2 and 13.8 ind. m^{-3}, respectively). *C. lividus* had peak abundances at Stns I01 and G99 (8.6 and 11.8 ind. m^{-3}; Fig. 3.7g). *C. mastigophorus*, which was less abundant than its similar-size congener *C. lividus*, was the least abundant species in the Ionian Sea, with a maximum abundance at Stns 907 and G99 (2.2 and 3 ind. m^{-3}; Fig. 3.7h).

The cluster analysis revealed three groups of stations characterized by their species composition (Fig. 3.8, Table 3.3):

- Cluster I (Stns C05, C03, G99, T04, I01, 907, T08, T02, T05, 004) is characterized by the striking dominance of *C. paululus* and much lower contributions of *C. arcuicornis*, *C. jobei*, *C. parapergens* and *C. pergens*;

- Cluster II (Stns C06, C07, C08, C09) is characterized by the co-dominance of *C. paululus* and *C. arcuicornis* (which together made up 60% of total *Clausocalanus* females) and by the contribution of *C. jobei*, *C. parapergens* and *C. pergens* that reached their highest abundance at these stations;

- Stn S02 differentiated from the other stations by having only four species: *C. lividus* (56.3% of female abundance), *C. furcatus* (20%), and *C. paululus* and *C. arcuicornis* (12.5% each).

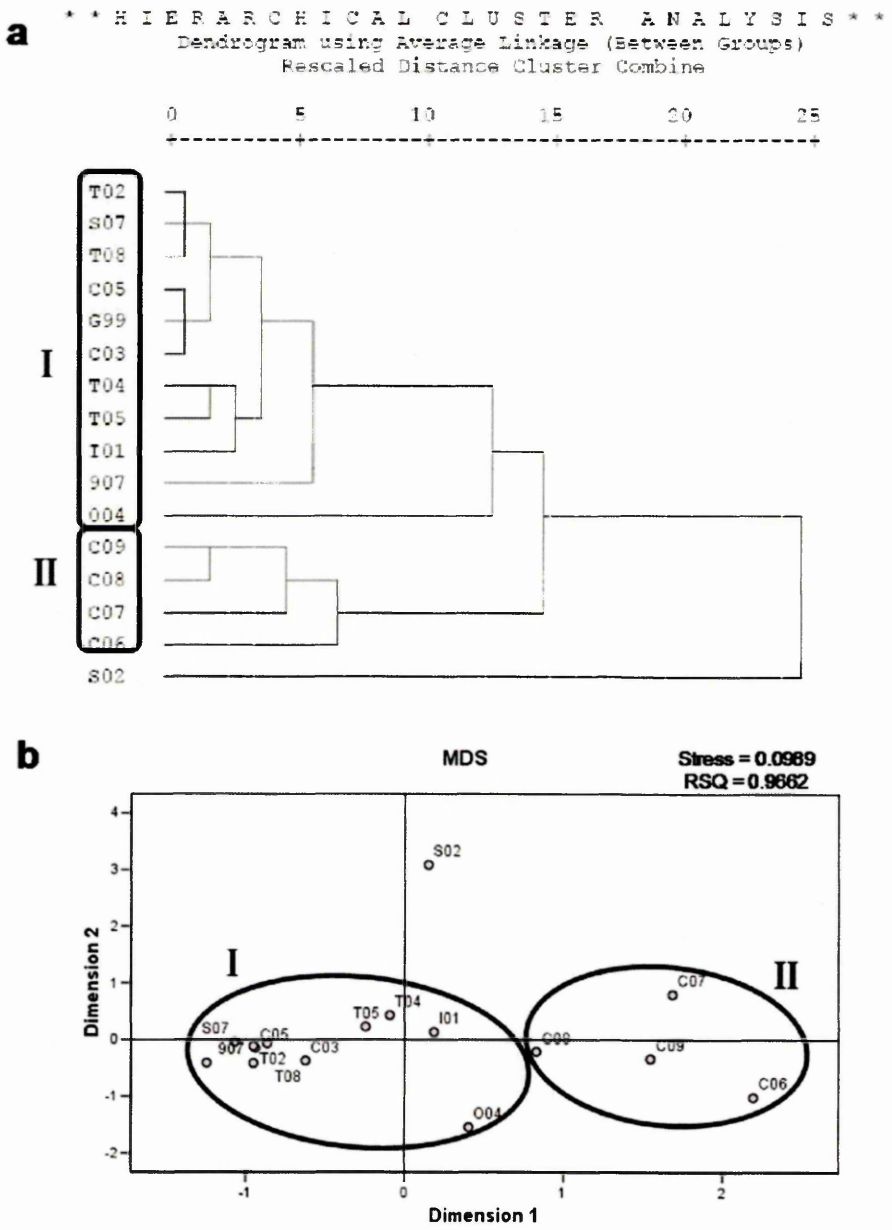


Figure 3.8. Results of species composition (relative contribution of each species females to total female abundance) statistical analysis. (a) Cluster analysis. (b) Non-metric multidimensional scaling. Station groupings are shown.

Table 3.3. *Clausocalanus* species composition (in terms of abundance, ind. m^{-3}) for each cluster in the Ionian Sea in spring 2002 (mean $\pm$ SD).

CLUSTER	I	II	Stn S02
C. paululus	37.6 ± 11.3	67.5 ± 10.2	12.5
C. pergens	4.1 ± 3.5	0.6 ± 1.2	0.0
C. furcatus	8.2 ± 5.3	6.2 ± 3.9	18.8
C. jobei	12.6 ± 4.5	2.7 ± 1.8	0.0
C. parapergens	9.3 ± 4.0	4.9 ± 2.1	0.0
C. arcuicornis	22.5 ± 4.6	7.6 ± 2.9	12.5
C. lividus	5.2 ± 5.7	8.3 ± 5.1	56.3
C. mastigophorus	0.6 ± 0.7	2.2 ± 1.4	0.0

3.1.3. Discussion

The Ionian Sea is a markedly oligotrophic basin, where the stratification of water masses with very different characteristics prevents a throughout mixing in winter and nutrient enrichment of the euphotic layer (Napolitano *et al.*, 2000). Very few studies in the past have provided information on zooplankton distribution in the basin (Greze, 1963; Vaissière and Seguin, 1980; Christou *et al.*, 1990). Surveys conducted in more recent years at the basin scale have shown that epipelagic mesozooplankton abundances in the open waters are very low in autumn (Mazzocchi *et al.*, 1997) and slightly higher in spring (Mazzocchi *et al.*, 2003) and show a strong vertical gradient from the upper 100 m down to 300 m depth.

In the spring of 2002, most zooplankton stations were grouped in two clear clusters, characterized by the presence of two different water masses: Modified Atlantic Water (MAW) and Ionian Surface Water (ISW), in accordance with the surface water mass characterization presented by Manca *et al.* (2006). The chlorophyll distribution retrieved by SeaWiFs (D'Ortenzio, 2003) confirmed the oligotrophic conditions in most of the basin (chlorophyll rarely higher than 0.2 μg

Chl *a* L^{-1}), but also highlighted a significant enhancement of surface autotrophic biomass offshore the Calabrian coast (north-western Ionian Sea). The "Calabrian bloom" (up to 0.8 μg Chl *a* L^{-1}) showed great temporal variability in the three years analyzed by D'Ortenzio (2003), however it was almost always present in March and disappeared in mid-April. This phenomenon was a novel feature for the basin, which appeared after the Eastern Mediterranean Transient (D'Ortenzio, 2003).

In the spring of 2002, *Clausocalanus* represented a relevant and often dominant component of the whole copepod assemblage in the upper 0-100 m layer. Also in other oligotrophic epipelagic regions, *Clausocalanus* has been reported as a dominant genus, e.g. in the Caribbean Sea (Webber and Roff, 1995) and in the Sargasso Sea (Paffenhöfer and Mazzocchi, 2003). In the Sargasso Sea, in summer, Paffenhöfer and Mazzocchi (2003) observed the predominance of juveniles in *Clausocalanus* assemblages but they used smaller mesh size (63 μm) which collected all copepodid stages more efficiently.

Clausocalanus were more abundant, and contributed more to total copepods, in the eastern-central side of the Ionian Sea, which was occupied by the ISW and was the most oligotrophic area of the basin. The lowest abundance and relative contribution of *Clausocalanus* were observed at those stations affected by the MAW and at those affected by the ISW with slightly higher fluorescence. The *Clausocalanus* assemblage was differentiated into three clusters of stations: 1) stations occupied by the oligotrophic ISW, where *C. paululus* dominated, 2) stations occupied by the MAW, where *C. arcuicornis* and *C. paululus* co-dominated, and 3) Stn S02 that showed the highest fluorescence values ("the Calabrian bloom") and a particular *Clausocalanus* assemblage where copepodids represented more than 90% of the total genus abundance, *C. lividus* was the most abundant species

and four species were not found at this station.

The small *C. paululus* was the most abundant species mainly in the eastern region occupied by the cold oligotrophic and salty ISW. The same species accounted for most of *Clausocalanus* abundances in other spring surveys conducted in the open Ionian Sea in the '90s (Mazzocchi *et al.*, 2003). The importance of *C. paululus* in the Ionian Sea seems to be related to the season. In fact, during the autumn, it was *C. furcatus* that prevailed in *Clausocalanus* associations all over the Eastern Mediterranean (Ionian included), being the most abundant copepod together with *Oithona plumifera* (Siokou-Frangou *et al.*, 1997).

In the spring of 2002, the two small species *C. pergens* and *C. furcatus* occurred at low abundance, the former only in the MAW region and in the Otranto Strait, while the second was recorded at all the surveyed stations but mainly in the warmer southern region. *C. arcuicornis* was an abundant species in the southern region occupied by the warmer, less salty, oligotrophic MAW; it outnumbered *C. jobei* and *C. parapergens*, but all three medium-sized species showed similar horizontal distribution. The large species *C. lividus* and *C. mastigophorus* occurred in low numbers; the former was more abundant than the latter but both species did not show clear distribution patterns due to their poor and sparce occurrence. Located offshore, the Calabrian coast, Stn S02 was dominated by *Clausocalanus* juveniles, suggesting that more eutrophic conditions at that site could sustain more intense *Clausocalanus* reproduction.

In synthesis, *Clausocalanus* was an important copepod genus in the spring epipelagic zooplankton throughout the Ionian Sea, but the species composition in this oligotrophic environment varied in accordance with the main hydrographical features occurring in the area (MAW, ISW and Calabrian bloom). The most abundant species in that period was *C. paululus*, which dominated the stations

with colder and more oligotrophic conditions.

3.2. Eutrophic conditions in the North Balearic Sea (Northwestern Mediterranean)

The Northwestern Mediterranean (NWMED) is the region were the most intense phytoplankton bloom occurs in the Mediterranean. It is one of the few mid-latitude basins where deep-water formation occurs in winter (Madec *et al.*, 1996). This region is strongly affected by wind gusts during winter and spring (Mistral, Tramontane), associated with intense cooling. Such conditions favor deep winter convection (chimney formation) that brings nutrients to the surface layer where low light inhibits photosynthesis. As soon as sunlight increases, an intense phytoplankton bloom develops which lasts for two weeks - one month in the end February - early May period and produces 15% of the total Mediterranean primary production (Antoine *et al.*, 1995). Biomass distribution is modulated by intense mesoscale activity and breakup of the deep-mixed patch (associated with the process of deep-water formation; Lévy *et al.*, 2000) and the North Balearic Front instabilities.

Restratification of the water column is a prerequisite for the onset of spring phytoplankton blooms in offshore waters of temperate regions (Sverdrup, 1953). However, the spring bloom in the northwestern Mediterranean (NWMED) starts before the seasonal stratification of the water column (Marty, 1993; Lévy *et al.*, 2000) and is very variable in its timing (André 1990, in Lévy 2000). Satellite SeaWiFS data revealed that this area is characterized by having the highest values of autotrophic biomass in the Mediterranean (D'Ortenzio, 2003). The modelling study of Lévy *et al.* (2000) showed the combined impact of mesoscale activity,

spring warming and wind gusts on the onset and decay of the spring phytoplankton bloom.

In the spring of 2003, two successive oceanographic cruises were conducted in the North Balearic Sea in the frame of the Italian programme NorBal, which was focused on the impact of deep water formation and the successive phases of spreading and restratification of the water column on biological productivity through a Lagrangian experiment. Lagrangian experiments, by following the same water mass using buoys, include both spatial and temporal variability permitting analysis at the same time of both different scales (space and time). The distribution of *Clausocalanus* species was analysed to assess the dynamics of these copepods in response to the temporal and spatial evolution of the spring phytoplankton bloom. During spring 2003, the phytoplankton community had high biomass (up to 17.1 x 10^6 cells L^{-1}) and very diverse composition, mostly dominated by small cells, mainly flagellates and diatoms (Iennaco, 2004).

3.2.1. Materials and methods

Two oceanographic cruises were conducted in the NWMED in the spring in 2003 during two legs of NorBal-4 (3-21 March) and one leg of NorBal-5 (21-25 April) cruises onboard the R/V *Urania* (Fig. 3.9). CODE (Coastal Ocean Dynamic Experiment) surface drifting buoys were launched at different times during the cruises (Table 3.4). The buoys were equipped with an Argos transmitter that automatically sends messages that are received by satellites in low-earth orbit (in this case the NOAA geostationary satellite in polar orbit) which in turn relay messages to ground stations that forward messages to processing centers who calculated buoys track and velocity (www.argos-system.org).

Mesozooplankton were collected at 22 stations in total (Table 3.4; Fig. 3.9)

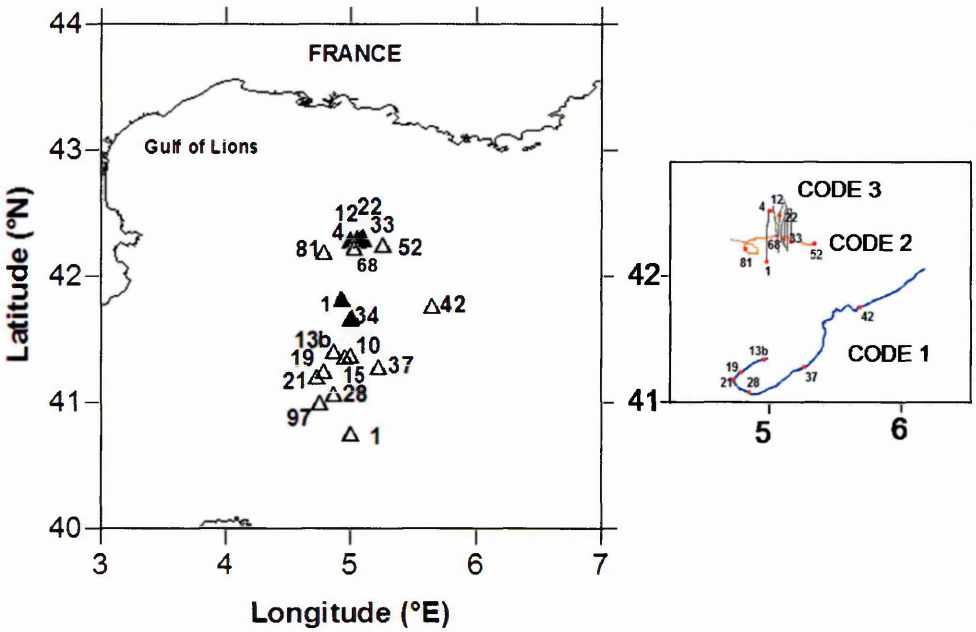


Figure 3.9. Area of zooplankton survey in the North Balearic Sea during NorBal-4 (white triangles) and NorBal-5 (black triangles) in the spring of 2003.

along three buoy tracks for monitoring the same water mass, as well as outside the track to obtain information on mesoscale variability that characterized the area. Vertical tows of a closing Nansen net (113 cm mouth diameter, 200 μm mesh size) were performed at three discrete depths (200-100, 100-50 and 50-0 m). Once onboard, mesozooplankton samples were immediately fixed and preserved in a buffered formaldehyde-seawater solution (4% final concentration).

In the laboratory, zooplankton were identified and counted under a dissecting microscope using a Bogorov chamber. *Clausocalanus* females and males were identified at the species level in three species (*C. paululus*, *C. pergens* and *C. furcatus*) while for the other species only females were identified at the species level according to Frost and Fleminger (1968), while the rest of the males and copepodids were identified only at the genus level (counts performed by M. G. Mazzocchi).

Frequent CTD casts were performed every day during NorBal-4 and NorBal-5

Table 3.4. Samples collected in the northwestern Mediterranean region during spring 2003. Collection time is reported in Coordinated Universal Time (UTC), a high-precision atomic time standard.

BUOY	Stations	DATE	TIME	COORDINATES		SONIC
		(dd/mm)	(UTC)	Lat.(°N)	Long.(°E)	DEPTH (m)
Leg 1						
-	NB4-01	06/03	10.29	42°14.99	06°15.02	2568
-	NB4-04	07/03	14.03	42°15.00	05°30.00	2321
-	NB4-10	08/03	13:20	41°22.48	04°59.99	2529
-	NB4-13b	09/03	9:08	41°24.48	04°51.97	2539
CODE-1	NB4-15	10/03	9:16	41°21.24	04°57.18	2537
CODE-1	NB4-19	11/03	3:24	41°15.04	04°47.00	2580
CODE-1	NB4-21	11/03	15:33	41°11.83	04°43.53	2600
CODE-1	NB4-28	12/03	11:30	41°04.32	04°51.70	2610
CODE-1	NB4-37	13/03	12:47	41°16.53	05°13.20	2530
Leg 2						
CODE-1	NB4-42	17/03	10:17	41°45.52	05°38.75	2480
CODE-2	NB4-52	18/03	10:51	42°14.88	05°14.95	2261
CODE-2	NB4-68	20/03	15.30	42°22.04	05°1.47	2054
CODE-2	NB4-81	21/03	11:08	42°11.61	04°47.32	2108
-	NB4-88	23/03	9.03	42°39.99	4°29.95	802
-	NB4-94	24/03	14.03	41°15.00	4°30.00	2616
-	NB4-97	25/03	7:10	40°59.99	04°44.95	2664
Leg 3						
CODE-3	NB5-01	18/04	21:36	40°44.98	05°00.01	2708
CODE-3	NB5-04	21/04	11:22	42°16.66	05°00.04	1952
CODE-3	NB5-12	22/04	9:02	42°16.93	05°03.03	2010
CODE-3	NB5-22	23/04	9:11	42°18.15	05°05.38	2046
CODE-3	NB5-33	24/04	12:07	42°17.29	05°05.89	2080
-	NB5-34	24/04	17:36	41°40.00	05°00.00	2423

cruises, but only those close to the time of mesozooplankton collection have been considered for the present analysis.

3.2.2. Results

Environmental parameters

Ocean colour images in the NWMED region during the surveyed period, showed remarkable mesoscale dynamics with eddy variability in the area (Fig. 3.10; D'Ortenzio, 2003). In mid-March, deep water formation and large phytoplankton bloom dynamics were recorded (Fig. 3.10a). At the end of March, the bloom covered the entire area (Fig. 3.10b) while in April the bloom was decaying (Fig. 3.10c). Vertical profiles of environmental data showed the three phases of

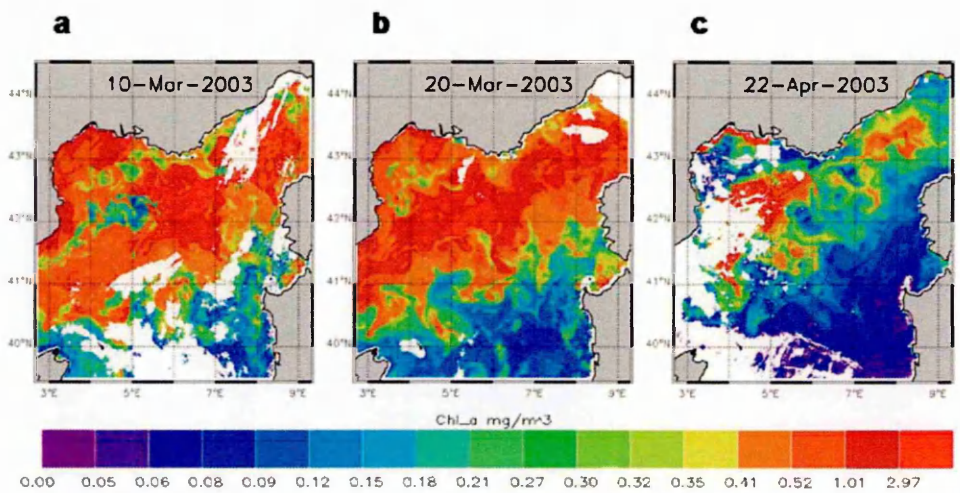


Figure 3.10. SeaWiFs images recorded in the NWMED region during spring 2003 (from D'Ortenzio, 2003). The blue 'hole' in a) corresponded to the deep water formation area while surrounding red areas indicated the occurrence of a large phytoplankton bloom.

the phytoplanktonic bloom (Fig. 3.11). At the onset of the bloom, during the second week of March 2003 (leg 1: Stns NB4-10 and NB4-13b before launching

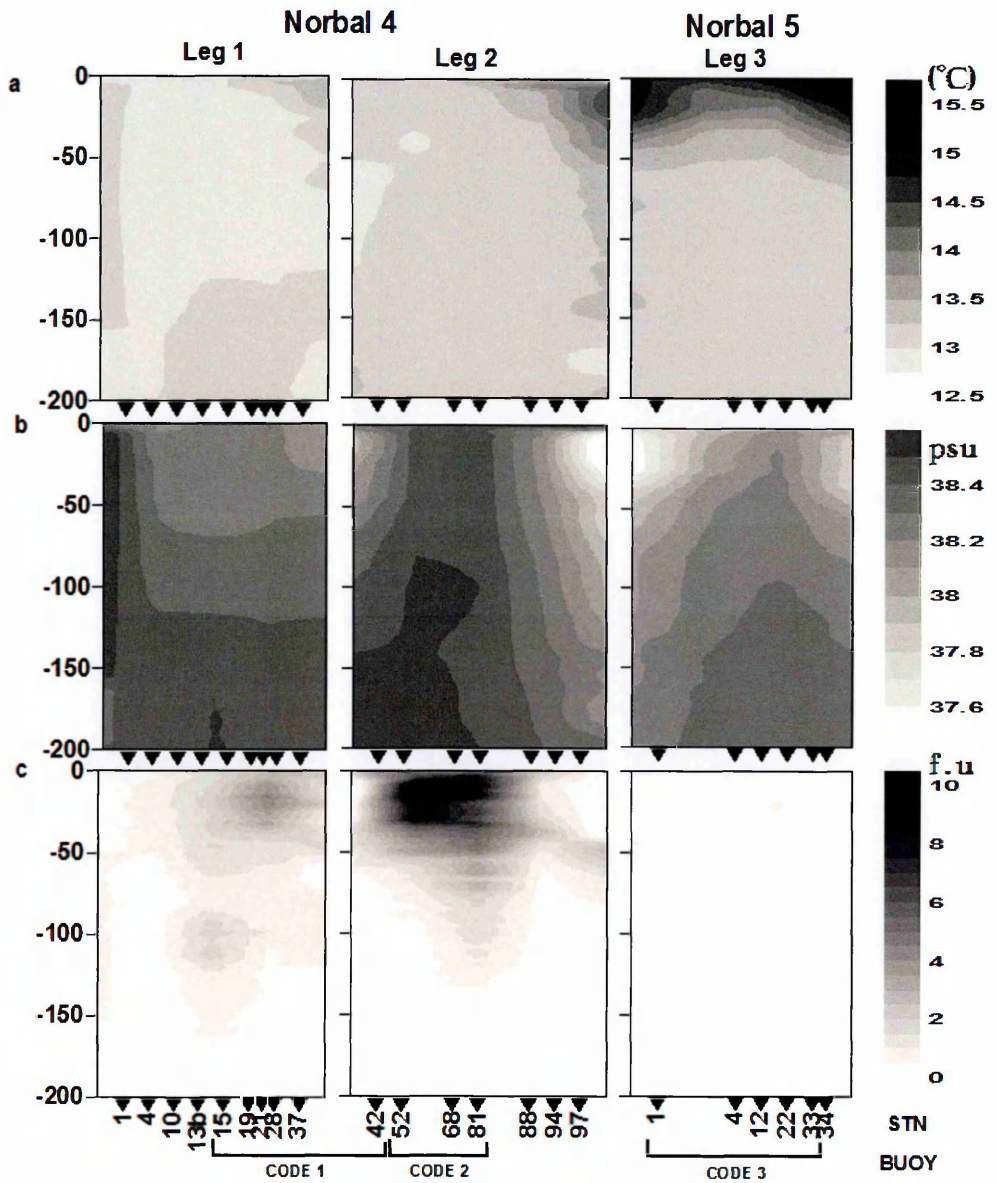


Figure 3.11. Water column structure during NorBal-4 (left) and NorBal-5 (right): temperature (a), salinity (b), and fluorescence (c) recorded by CTD casts in the upper 200 m in spring 2003.

the buoy and from Stns NB4-15 to NB4-37 on CODE 1 track), the water mass was cold (Fig. 3.11a), salinity was high (Fig. 3.11b) and autotrophic biomass was concentrated in the upper 70 m (Fig. 3.11c). It must be noticed that few hours after sampling on 12 March, surface temperature increased and salinity decreased, suggesting the intrusion of a different water mass. Also phytoplankton concentration increased at depth layers (Iennaco, 2004). All these observations indicate that the water mass followed by CODE-1 deepened and that stations visited successively were occupied by a different water mass (Dr. B. Buongiorno-Nardelli, Gruppo di Oceanografia da Satellite ISAC-CNR, personal communication).

During the bloom, in the third week of March (leg 2: Stn NB4-42 on CODE-1 track, Stn NB4-52 and NB4-81 on CODE-2 track and Stn NB4-97 outside the track), the temperature was higher than on the previous leg and the water column started to stratify (Stn NB4-97). The highest fluorescence was recorded during this leg and was concentrated in the upper 50 m (Fig. 3.11c). In the last week in April (leg 3: Stns NB5-01 to NB5-34), the water column was stratified, and a clear thermocline was recorded in the upper 50 m layer (Fig. 3.11a). On days 19 and 20 (between Stn NB5-01 and NB5-04), strong Tramontane wind in the area slightly destabilized the thermocline that was restored during the next days. The lowest fluorescence values were recorded during this period (Fig. 3.11c).

Cluster and non-metric multidimensional scaling (MDS) revealed four clusters of stations (Fig. 3.12, 3.17a):

- Cluster I grouped together those stations where stratification was noticed in the upper layer (Stns NB4-97, NB5-1, 34). Water temperature was the highest (14.2°C) while salinity was the lowest (37.8 psu). Fluorescence was also low (0.34 f.u.).

- Cluster II grouped together the upper 50 m of those station where the

maximum fluorescence values were recorded (Stns NB4-52, 68, 81; 6.9 f.u.). Temperature (13.1°C) and salinity (38.4 psu) were similar to those recorded in the successive clusters.

- Cluster III was formed by the upper layer of those stations where the minimum fluorescence values were recorded (Stns NB5-4, 12, 22, 33; 0.24 f.u.).
- Cluster IV grouped all the remaining layers and stations, characterized by low temperatures (13.1°C), high salinity (38.3 psu) and mid fluorescence (0.8 f.u.).

Clausocalanus

In the integrated 200 m water column, *Clausocalanus* abundance showed a general increasing trend from the beginning to the end of the survey, with increasing variability at the same time. The overall mean was 625.2 ind. m^{-3} ($\pm$413.8)(Fig. 3.13a, Table 3.5). On average, *Clausocalanus* represented 59.1% of the total copepods ($\pm$8.9) ranging from 40.4 to 73.2% (Fig. 3.13b). *Clausocalanus* contribution to total copepod abundance slightly increased with time in March. In April, along the buoy track, the highest contribution recorded (76.2% at Stn NB5-01) was followed by much lower values, which regularly increased in a few days. *Clausocalanus* population was mainly represented by copepodids (58.8% of total *Clausocalanus*). Their contribution was quite stable during the track of first buoy while tended to increase during the tracks of the second and third buoys. Males made up, on average, 12.4$\pm$5.4% of total *Clausocalanus* abundance and showed their highest contributions during leg 2 (Fig. 3.13a, Table 3.5).

Clausocalanus females were dominated by far by *C. pergens* (Fig. 3.13d). Females of *C. pergens* showed a remarkable variability in body size from 0.87-

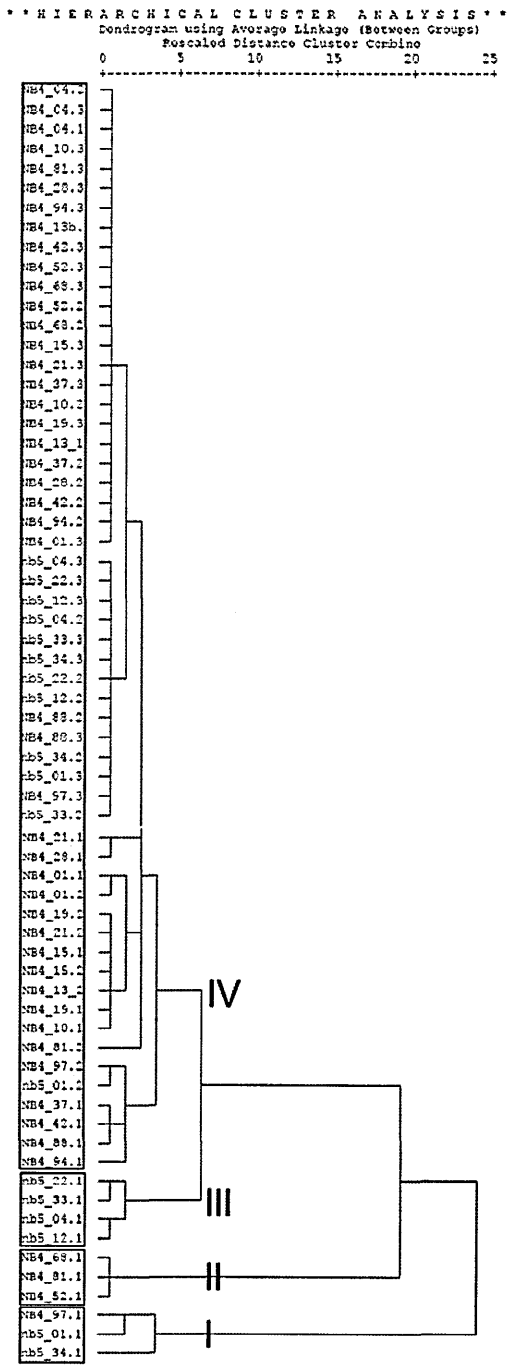


Figure 3.12. Dendrogram of environmental parameters (temperature, salinity and fluorescence) recorded at the three different depth layers where mesozooplankton samples were collected (.1, 0-50 m; .2, 50-100 m; and .3, 100-200 m). Spring 2003 in the North Balearic Sea.

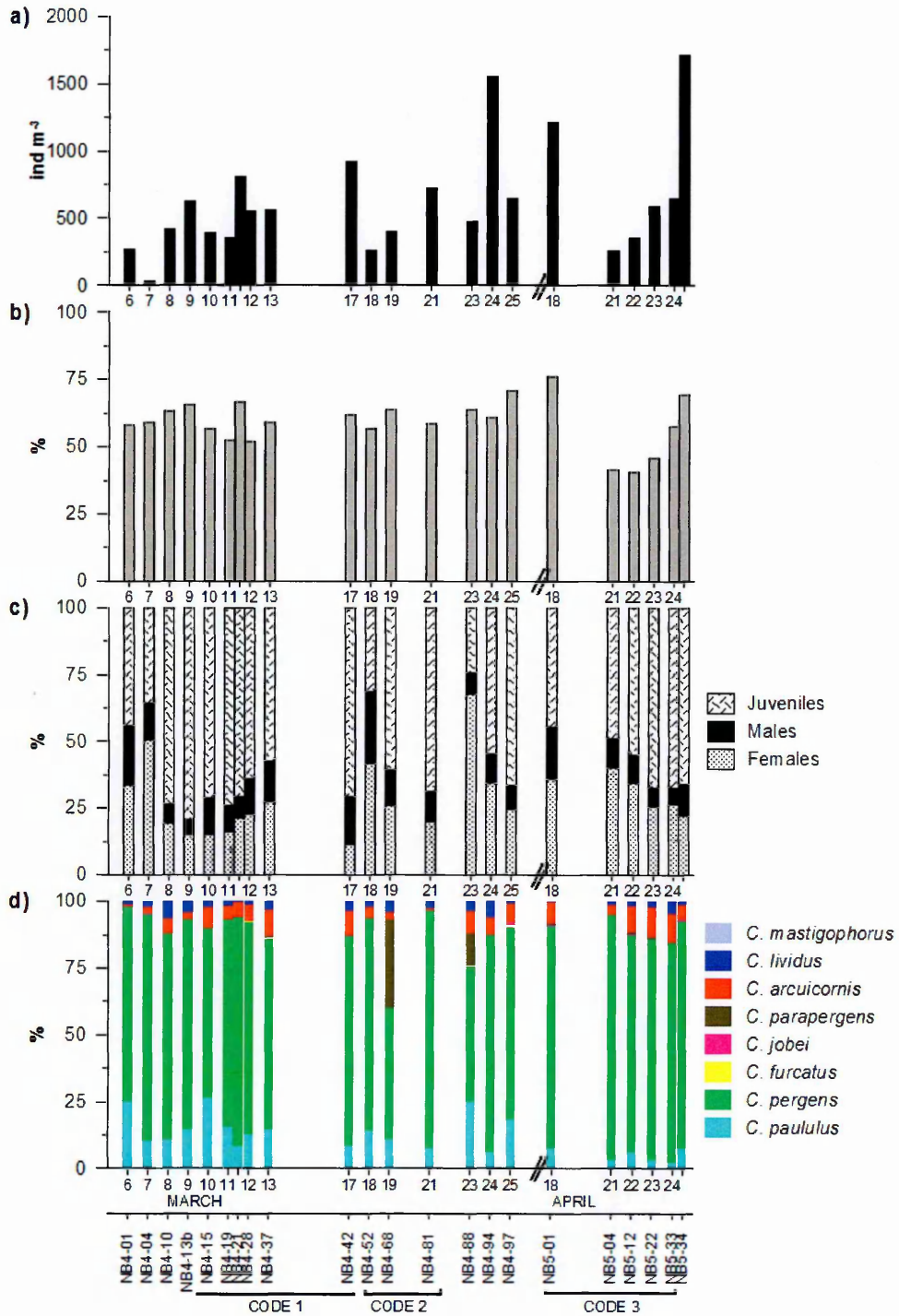


Figure 3.13. *Clausocalanus* abundance (a), relative contribution (b), population structure (c) and species relative contribution (adult females)(c) in the integrated 0-200 m in the NWMED in spring 2003.

Table 3.5. *Clausocalanus* abundance, relative contribution and population structure in the integrated 0-200 m in the northwestern Mediterranean during spring 2003.

	LEG 1 (8-12 March)	LEG 2 (17-25 March)	LEG 3 (18-24 April)	Whole period
Genus abundance (ind. m^{-3})				
mean	445.9	710.1	795.2	625.2
SD	224.5	430.2	560.2	413.8
min	29.1	260.8	257.1	29.1
max	807.3	1550.8	1714.9	1714.9
Genus % of total copepods				
mean	59.2	62.3	55.1	59.1
SD	5.3	4.5	15.2	8.9
min	51.7	56.5	40.4	40.4
max	66.6	70.7	76.2	76.2
% of females to total *Clausocalanus*				
mean	24.7	32.3	30.9	28.8
SD	11.4	18.4	7.1	13.1
min	15.2	11.5	22.4	11.5
max	50.6	67.8	40.3	67.8
% of males to total *Clausocalanus*				
mean	12.1	13.9	11.0	12.4
SD	5.2	6.5	4.6	5.4
min	5.8	8.0	6.4	5.8
max	22.5	26.7	19.4	26.7
% of juveniles to total *Clausocalanus*				
mean	63.2	53.7	58.1	58.8
SD	14.9	18.6	10.0	15.0
min	35.4	24.2	44.5	24.2
max	79.0	70.4	67.2	79.0

Table 3.6. Females of *Clausocalanus* species relative contribution in the northwestern Mediterranean during spring 2003. For each species, mean ($\pm$SD) and range (minum-maximum) during each period are reported.

Species	LEG 1 (8-12 March)	LEG 2 (17-25 March)	LEG 3 (18-24 April)	Whole period
C. paululus	15.2 (6.3)	12.7 (6.7)	4.7 (2.5)	11.6 (7.0)
	(8.3-26.3)	(6.1-24.6)	(1.9-7.4)	(1.9-26.3)
C. pergens	76.8 (6.8)	71.5 (15.4)	84.5 (3.7)	77.2 (10.7)
	(63.4-85.5)	(49.4-89.0)	(81.7-91.8)	(49.4-91.8)
C. furcatus	0.0 (0.1)	0.2 (0.3)	0.0 (0.1)	0.1 (0.2)
	(0.0-0.2)	(0.0-0.7)	(0.0-0.2)	(0.0-0.7)
C. jobei	0.1 (0.2)	0.2 (0.4)	0.2 (0.2)	0.2 (0.3)
	(0.0-0.7)	(0.0-1.1)	(0.1-0.6)	(0.0-1.1)
C. parapergens	0.0 (0.1)	6.4 (12.5)	0.1 (0.2)	2.1 (7.3)
	(0.0-0.4)	(0.0-33.1)	(0.0-0.6)	(0.0-33.1)
C. arcuicornis	5.3 (2.9)	5.7 (3.1)	8.3 (3.0)	6.2 (3.1)
	(1.0-10.3)	(0.8-9.5)	(3.7-11.4)	(0.8-11.4)
C. lividus	2.6 (1.8)	3.4 (1.6)	2.1 (1.5)	2.7 (1.6)
	(0.5-6.4)	(0.9-5.9)	(0.5-4.9)	(0.5-6.4)
C. mastigophorus	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)	0.0 (0.0)
	(0.0-0.0)	(0.0-0.1)	(0.0-0.0)	(0.0-0.1)

1.07 mm, similar to that reported by Frost and Fleminger (1968), and approaching the medium size of *C. parapergens*. *C. pergens* accounted for $77.2 \pm 10.7\%$ during the whole period. At all stations but two, it made up >70% of total *Clausocalanus* females, increasing from leg 1 to leg 3 (Table 3.6). It was followed by *C. paululus*, which mainly contributed in March (leg 1) and *C. arcuicornis*, which mainly contributed in April (leg 3). *C. lividus* was present during the whole period with similar very low contributions ($2.7 \pm 1.6\%$). *C. jobei* was very scarce ($0.2 \pm 0.3\%$), while *C. parapergens* made up a relevant contribution only at Stn NB4-68 and NB4-88 (33.1% and 11.5%, respectively). *C. mastigophorus* was sparcely recorded with only a few indiviuals.

Looking at the temporal variability of *Clausocalanus* species in spring 2003 in the NWMED (Fig. 3.14 and Fig. 3.15), it can be noted that the dominant *C. pergens* and the other most abundant species, *C. paululus*, *C. arcuicornis* and *C. lividus*, showed very similar temporal and spatial patterns with a general increasing trend of abundance from early March to late April (Fig. 3.14). *C. paululus* was more abundant than *C. arcuicornis* in March, while the opposite occurred in April.

The most abundant *Clausocalanus* species were concentrated in the upper 50 m during most of the studied period. Such species occurred deeper when in March the tracked water mass deepened (Stn NB4-15) and after a strong mixing event in April (Stn NB5-04). After that, species population rose up to the upper layer (Fig. 3.15).

Cluster and MDS analysis based on *Clausocalanus* species composition during the surveys, revealed that most of stations and layers presented similar *Clausocalanus* assemblages (due to the large dominance of *C. pergens*) except on three occasions (Fig. 3.16, 3.17b):

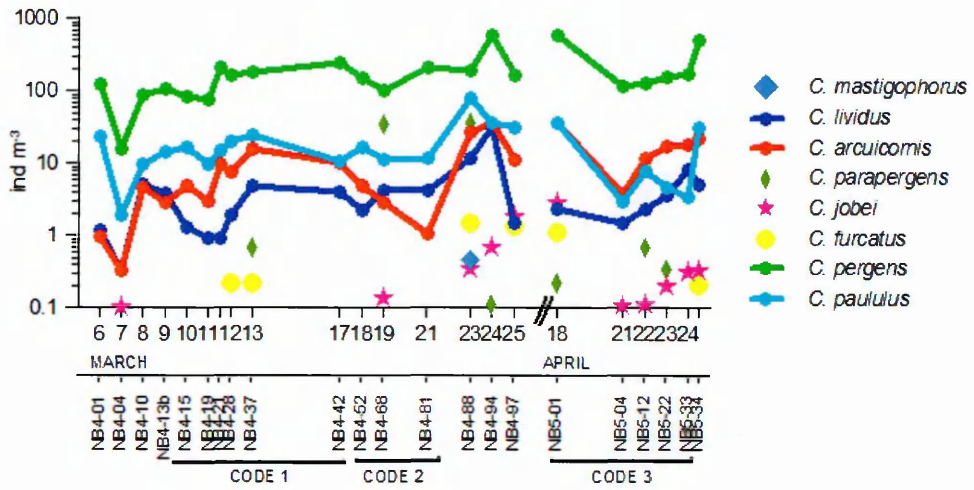


Figure 3.14. *Clausocalanus* female abundance integrated in the upper 200 m in the NWMED in spring 2003. Sampling dates are joined with lines in those species that were presented at all stations, sporadic presence are indicated by isolated symbols.

- At Stn NB4-68 in the 50-200 m where *C. parapergens* dominated (73% and 57% at 50-100 and 100-200 m layers);
- At Stn NB4-88 (50-100 m) where *C. paululus*, *C. arcuicornis* and *C. pergens* co-dominated (31.3%, 25.3% and 24.0%, respectively) and were followed by *C. lividus* (12.0%);
- At Stn NB4-97 (0-50 m) where *C. jobei* and *C. furcatus* (absent or almost absent in all the other stations) represented 4.2% and 2.6% of *Clausocalanus* females, respectively.

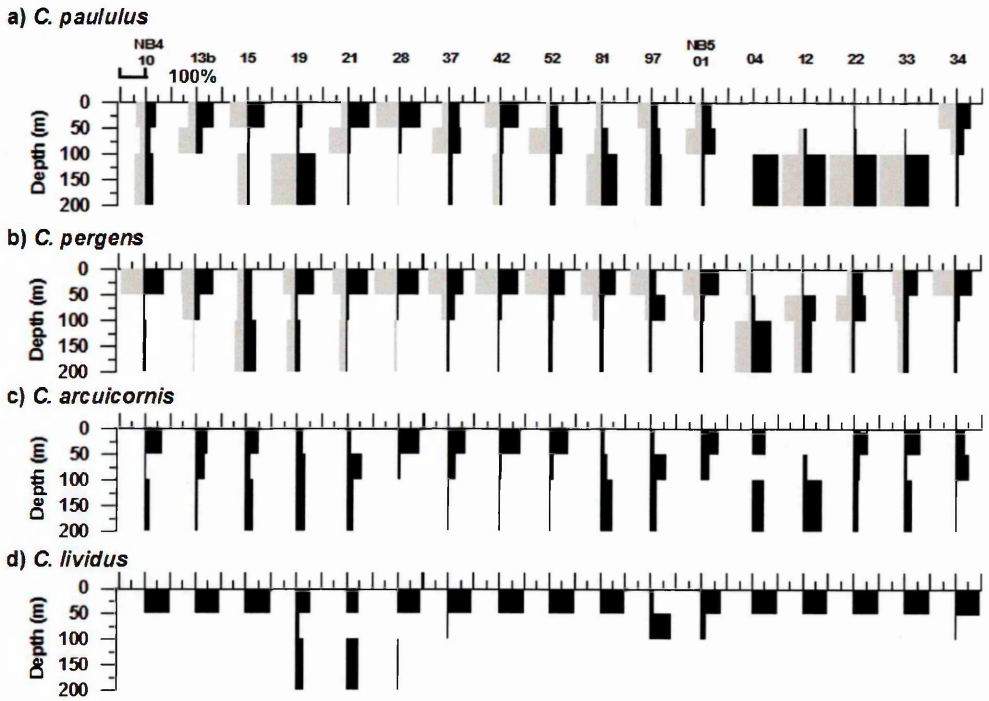


Figure 3.15. *Clausocalanus* females (black) and males (when data available; grey) vertical distribution in the NWMED in spring 2003.

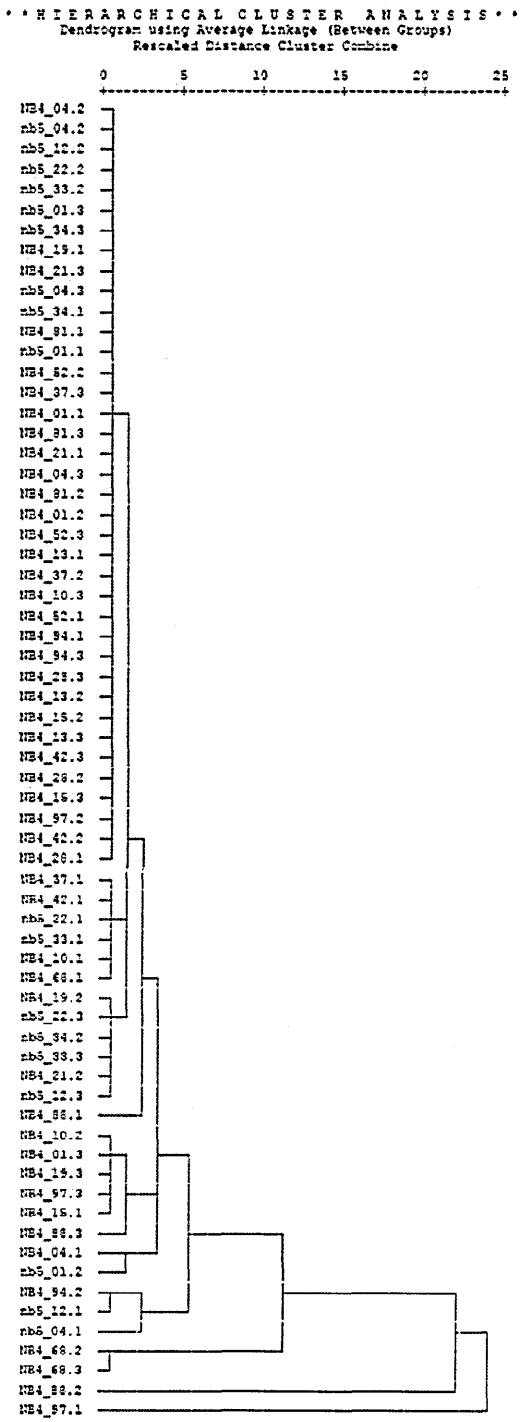


Figure 3.16. Dendrogram of *Clausocalanus* assemblages occurring at the three different depth layers where mesozooplankton samples were collected (.1, 0-50 m; .2, 50-100 m; and .3, 100-200 m). Spring 2003 in the North Balearic Sea.

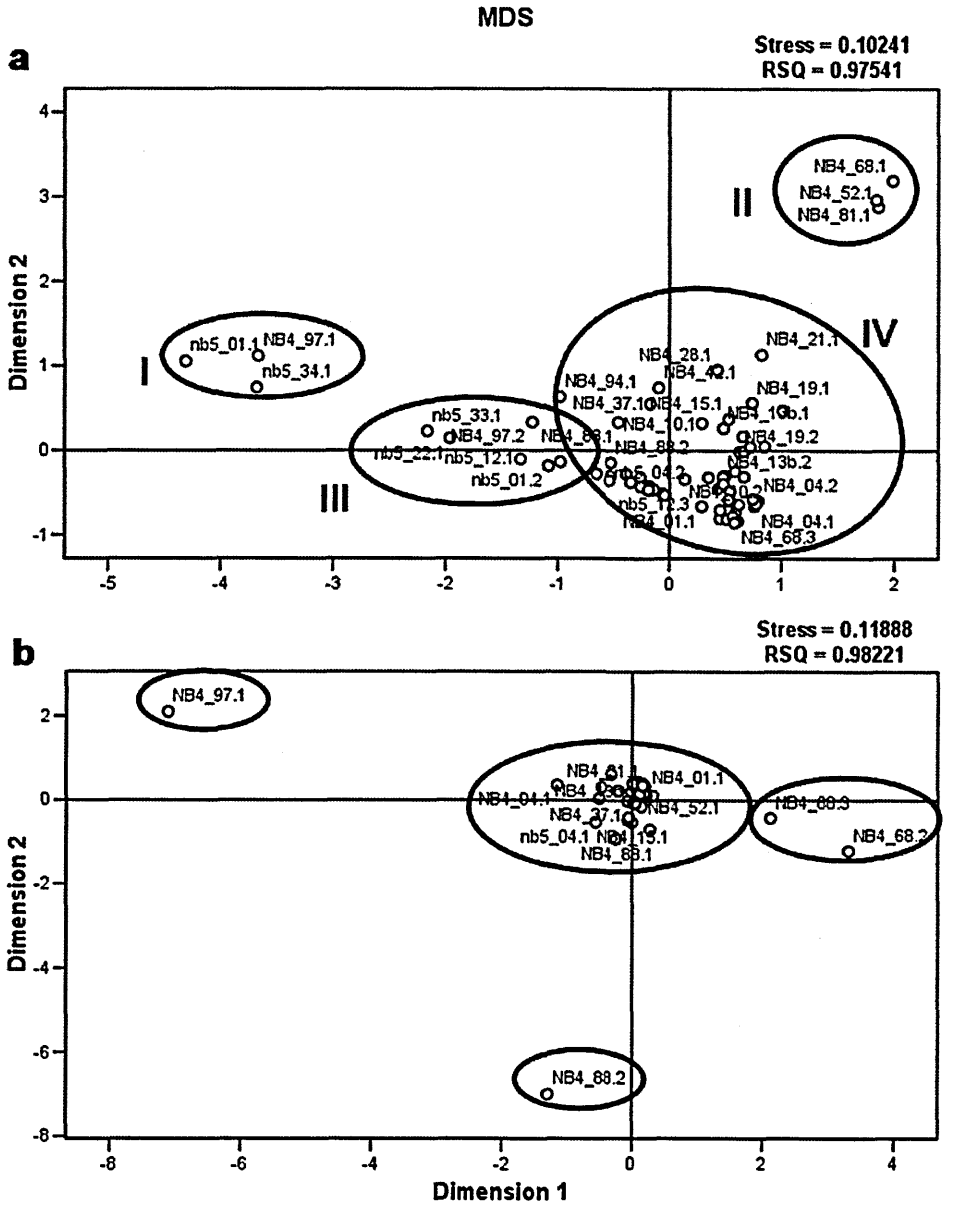


Figure 3.17. Non-metric multidimensional scaling of environmental parameters (temperature, salinity and fluorescence) (a) and *Clausocalanus* assemblages (b) encountered in the North Balearic Sea in spring 2003.

3.2.3. Discussion

The spatial and temporal distributions of *Clausocalanus* species were described in the North Balearic Sea in spring along the trajectories of three different buoys that were launched at different times and locations to follow the development and the distribution of an intense phytoplankton bloom.

Several processes are responsible for the variability of the spring phytoplankton bloom in the Northwestern Mediterranean (NWMED) according to Lévy *et al.* (2000). In March 2003, Ekman and dynamical restratification caused the bloom to start earlier in the season, before thermal restratification that occurred at the end of March. When thermal restratification occurred, fluorescence values were very low, indicating that the phytoplankton bloom had already decayed, probably due to nutrient depletion in the photic zone. Phytoplankton composition and abundance in the NWMED in the spring of 2003 was analysed by Iennaco (2004). The community was very diverse (c. 130 species) and mostly dominated by small cells (2-5 μm)(Iennaco, 2004). In March, small non colonial diatoms, small flagellates (ultra-pico plankton) and coccolithophores dominated, then large colonial diatoms appeared, characteristic of bloom conditions. In April, phytoflagellates and small coccoids dominated and small dinoflagellates became significantly more important. In March, phytoplankton was concentrated in the 0-20 m layer and gradually increased up to fourfold, with a major peak at 30 m, then it returned to the pre-bloom situation in late March. In April, phytoplankton concentration was half that observed in early March and was restricted to the upper 25 m. According to the phytoplankton community encountered along the survey, the stations visited during the first buoy track corresponded to the onset or medium phase of a bloom, those visited during the second buoy track corresponded to the peak of a bloom and those visited during the third buoy track corresponded to the end of

a bloom (Iennaco, 2004).

Mesozooplankton communities were dominated by *Clausocalanus*, which was mainly represented by copepodids indicating continuous recruitment throughout the area in that season, as also observed by Razouls and Kouwenberg (1993) in the offshore area of the Gulf of Lions and by Andersen *et al.* (2001b,a) in offshore waters of the Ligurian Sea. *C. pergens* outnumbered all other species as adults. This species was concentrated in the upper 50 m layer except when mixing took place and it occupied the whole water column. The highest abundances were observed in late March and mid-April, at the end of the phytoplankton bloom when phytoflagellates and small dinoflagellates were more abundant and the water column was in stratified calm conditions. Small scale turbulence enhances encounter rates between copepods and prey (Kiørboe and Saiz, 1995) but in such conditions *C. pergens* might not compete with filter feeding copepods. In calm conditions, during the season of its maximum occurrence, *C. pergens* may be favoured over other small calanoids likely due to ability in exploiting small patches of food items, as recently hypothesized for *C. furcatus* (Uttieri, 2006).

Adult *C. pergens* females showed a wide size range but that was not observed in males. Largest females reached the size of small *C. parapergens* females. Large *C. pergens* specimens were also observed by Williams and Wallace (1975) in the North Atlantic Ocean. In the Ligurian Sea, Andersen *et al.* (2001a) reported *Clausocalanus* among the most abundant copepods in May, constituted by *C. pergens*/*C. parapergens*, which suggests uncertainty in taxonomic identification likely due to size variability as in our observations. The body size distribution of adult females is sometimes bimodal or polymodal indicating that females with probable different origins co-occur. Overwintering populations can persist in spring and mix with adults of successive generation, which would be smaller because a

decrease in body size is generally observed with increase of environmental temperature. Also development time becomes shorter when environmental temperature increases. Development and growth are somewhat decoupled and development is more temperature sensitive than growth, hence size changes with changing temperature. Changes in temperature contribute to overlapping in the body size of the cohorts (Mauchline, 1998). When observing *C. arcuicornis* populations in the Gulf of Marseille, Gaudy (1972) noticed a reduced size in individuals developed in warm autumnal waters in comparison with larger individuals developed during the cold winter period. During our surveys in the spring of 2003, large range of body size in *C. pergens* was noticed since the first station, and it was the only species showing such feature.

Short-term changes observed in the vertical distribution of *Clausocalanus* could have been due to direct (mechanical) or indirect (dilution of food environment) causes related to the occurrence of strong winds in the area. Such meteorological events and the generated Ekman pumping, induced a deepening of the mixed layer and a dilution of the phytoplankton biomass in the water column. Abrupt changes in the vertical distribution of small-size zooplankton and in community composition was also observed by Andersen *et al.* (2001b) in the Ligurian Sea as a consequence of strong wind events in a late spring.

In synthesis, in the spring eutrophic conditions occurring in the northwestern Mediterranean in 2003, the copepod assemblages in the upper 200 m water column were dominated by *Clausocalanus*, which in turn were mostly represented by *C. pergens*. *C. paululus*, *C. arcuicornis* and *C. lividus* occurred at tenfold lower abundance than *C. pergens*. All four species were mainly concentrated in the upper 50 m layer and deepened as a probable consequence of wind-induced mixing during strong meteorological events. The remaining four *Clausocalanus* species

occurred only sporadically. The *Clausocalanus* assemblage was more diverse when heterotrophic phytoplankton was more important and the thermocline appeared. The omnivorous *Clausocalanus* species seem to be successfully adapted to exploit the stratified water column.

3.3. Comparison of *Clausocalanus* distribution in oligotrophic and eutrophic regimes

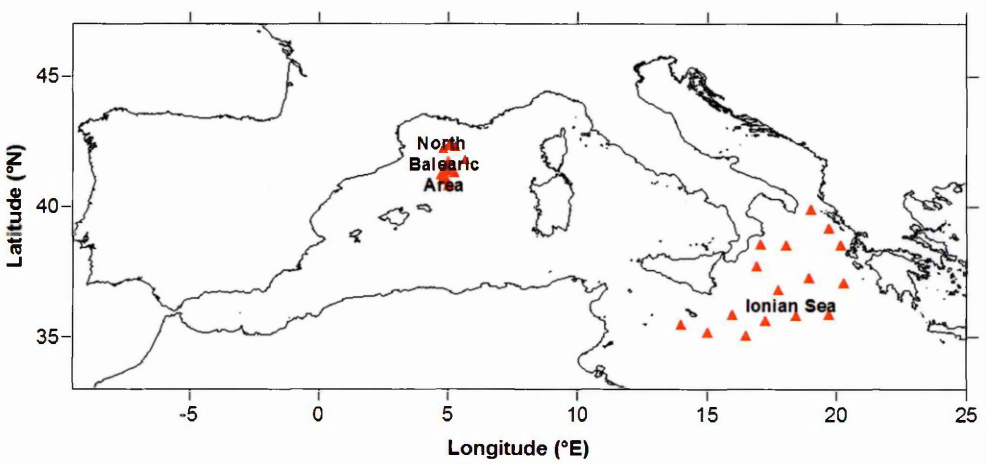


Figure 3.18. Surveyed areas in the Mediterranean Sea for the present *Clausocalanus* study.

The surveys conducted in the Ionian Sea (EMED) and in the North Balearic Sea (NWMED)(Fig. 3.18) allowed to compare the spring distribution of *Clausocalanus* in open environments with very different trophic conditions. The comparison that is presented here is based on abundance and species composition integrated in the upper 100 m of the water column. In the same spring period in two successive years, the upper 100 m water column in the EMED (2002) and in the NWMED (2003) differed in temperature (about 2.3°C warmer the EMED) and in the estimated quantity of autotrophic biomass (1.2 f.u. richer the NWMED).

In the oligotrophic Ionian Sea, *Clausocalanus* abundance was tenfold lower on average than in the eutrophic North Balearic Sea (Fig. 3.19a). However, notwithstanding this remarkable difference, this genus accounted for similarly relevant percentages of copepod abundance in both regions ($34.7 \pm 14.3\%$ in the Ionian Sea, $38.8 \pm 16.1\%$ in the North Balearic Sea) (Fig. 3.19b).

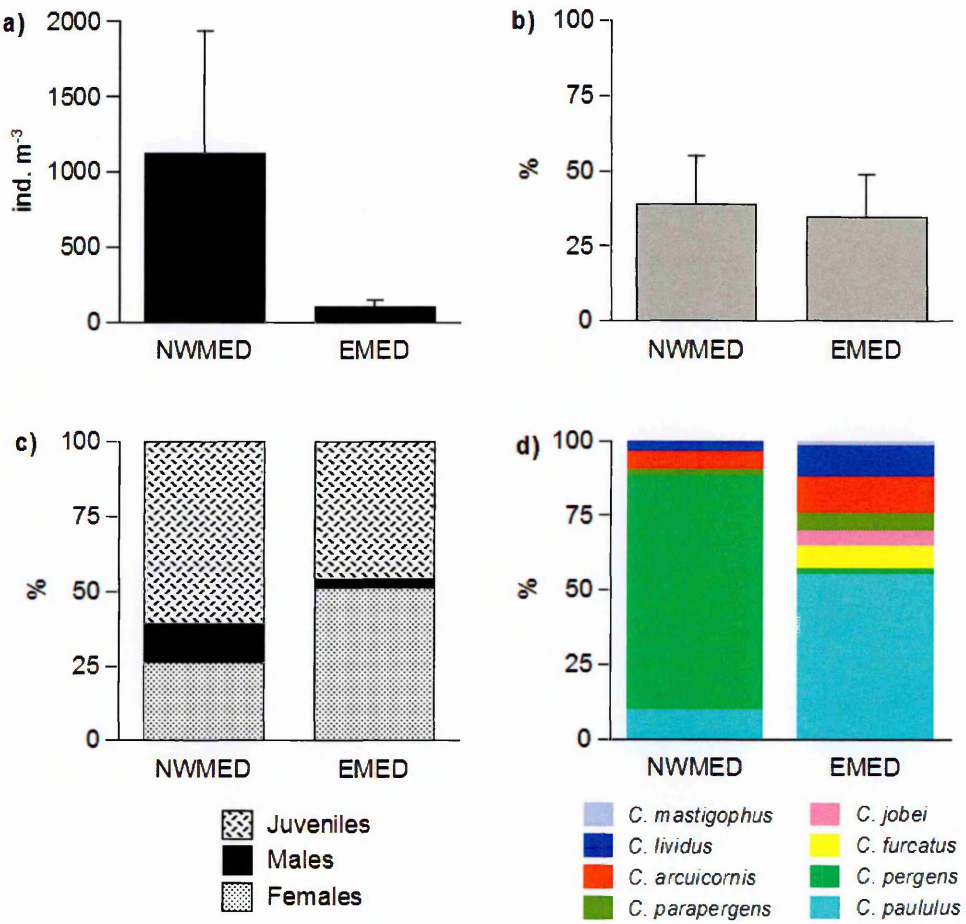


Figure 3.19. Comparison of *Clausocalanus* abundance and composition in spring conditions in two different trophic regimes: eutrophy in the northwestern Mediterranean (NWMED, North Balearic Sea, March-April 2003), oligotrophy in the Eastern Mediterranean (EMED, Ionian Sea, March-April 2002). Abundance (a), percentage contribution to total copepods (b), population composition (c) and species composition (d) in the upper 100 m water column.

In oligotrophic conditions, adult females and juveniles contributed similarly to population structure and males were very scarce, while in eutrophic conditions, juveniles dominated and males were more representative (Fig. 3.19c). This difference in population structure among sites was significant (ANOVA, $p \leq 0.001$).

At the species level, *C. paululus* dominated in oligotrophic conditions (55.4 $\pm$ 20.3%), while *C. pergens* dominated in eutrophic conditions (78.2 $\pm$ 11.2%). The other six Mediterranean *Clausocalanus* species make up all together 43.0% of adult females in the EMED and only 11.8% in the NWMED. While *C. arcuicornis* and *C. lividus* were representative in both regions, *C. furcatus*, *C. jobei* and *C. parapergens* contributed only in oligotrophic conditions.

Significant differences were observed between sites in *C. pergens* abundances (Fig. 3.20; t-test, $p \leq 0.001$), but not in *C. paululus* (t-test, $p \geq 0.05$). The third and fourth most abundant species in both regions were *C. arcuicornis* and *C. lividus*, which were more abundant in the NWMED than in the EMED (16.6 and 6.2ind. m^{-3} in the NWMED, and 6.3 and 3.9 ind. m^{-3} in the EMED, respectively) but significant difference between sites was observed only for *C. arcuicornis* (t-test, $p \leq 0.001$). *C. furcatus*, *C. jobei* and *C. parapergens* were significantly more abundant in the EMED (3.5, 2.9 and 3.1 ind. m^{-3}, respectively) and only occasionally recorded in the NWMED (0.2, 0.7 and 0.2 ind. m^{-3}, respectively)(t-test, $p \leq 0.001$). *C. mastigophorus* was by far the least abundant *Clausocalanus* species in both regions, especially in the NWMED where it was only observed in one station (0.04 $\pm$ 0.2 ind. m^{-3} in the NWMED, 0.9 $\pm$ 0.8 ind. m^{-3} in the EMED).

In synthesis, this comparative analysis highlighted the importance of the smallest *Clausocalanus* species in the spring epipelagic copepod assemblages, both in the Ionian Sea (*C. paululus*) and in the North Balearic Sea (*C. pergens*). The two species co-occurred in both regions, but their distribution patterns showed clearly

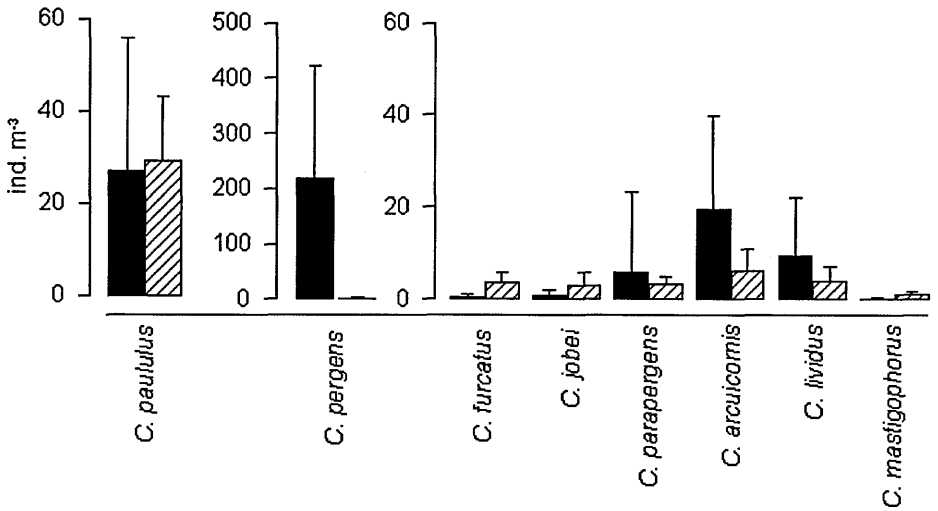


Figure 3.20. *Clausocalanus* species abundance in two different trophic conditions: the eutrophic North Balearic Sea (NWMED)(black histograms) and the oligotrophic Ionian Sea (EMED)(striped histograms).

that *C. paululus* prevails in oligotrophic conditions, while *C. pergens* prevails in eutrophic conditions.

CHAPTER 4

Latitudinal distribution in the Atlantic Ocean

In the Atlantic Ocean, the Atlantic Meridional Transect (AMT) programme performs biological, chemical and physical oceanographic research during the passage of the RRS *Discovery* between the UK and Cape Town or the return passage of the RRS *James Clark Ross* between the UK and the Falkland Islands. This transect is up to 13,500 km long and crosses a wide range of ecosystems: from subpolar to tropical, from eutrophic shelf areas and upwelling systems to oligotrophic mid-ocean gyres. Starting in 1995, the scientific aims of the AMT programme are assessing mesoscale to basin scale phytoplankton processes, the functional interpretation of bio-optical signatures and the seasonal, regional and latitudinal variations in mesozooplankton dynamics (Robins and Aiken, 1996). During the second part of the programme (2002-2006) aims were broadened to address a suite of cross-disciplinary questions concerning ocean plankton ecology and biogeochemistry and their links to atmospheric processes. Such objectives included the determination of how 1) the structure, functional properties and trophic status of the major planktonic ecosystems vary in space and time, 2) physical processes control the rates of nutrient supply, including dissolved organic matter, to the planktonic ecosystem, and 3) the atmosphere-ocean exchange and photodegradation influence the formation and fate of organic matter (http://www.pml.ac.uk/amt/index.htm).

Dr. R. Harris invited me to participate to the AMT programme to contribute

to hypothesis 1: "The size spectra, and mineralisation capacity of planktonic organisms are major determinants of CO_2 and organic matter export to the atmosphere and deep water" (lead investigators: Dr. X. Irigoien, AZTI and Dr. R.P. Harris, PML).

The study of *Clausocalanus* species distribution along the AMT latitudinal transect combined with *in situ* egg production experiments (reported in Part II) was aimed at 1) investigating *Clausocalanus* niches over a broad latitudinal scale and comparing them with those distributions observed in the Mediterranean Sea, and 2) comparing reproductive parameters with those recorded in the Mediterranean Sea and to further estimate secondary production of this important genus along a latitudinal transect.

The AMT-15 track between the UK and South Africa spans the UK continental margin, the Porcupine and Iberian Plain, the Canary Basins and the Cape Verde plateau in the North East Atlantic, the Mid-Atlantic Ridge, the Brazil basin, the Mid-Atlantic Ridge, the Walvis Ridge and the Cape basin.

According to global data set of the seasonal CZCS seasurface chlorophyll fields analysed by Longhurst *et al.* (1995); Longhurst (2006), there is a biogeochemical partitioning of the world oceans into different provinces. During AMT-15, eight provinces were crossed (Fig. 4.1).

- NECS Northeast Atlantic Continental Shelf. From the narrow shelf of western France, north across the British shelf and North Sea, and including also the Baltic Sea where there is seasonal ice cover.

- NADR North Atlantic Drift. Comprises the North Atlantic Current, lying south of the Oceanic Polar Front and of the Subarctic Front over the Iceland-Faeroe Ridge. To the south, the separation from the northern limb of the subtropical gyre lies at ≈40°N.

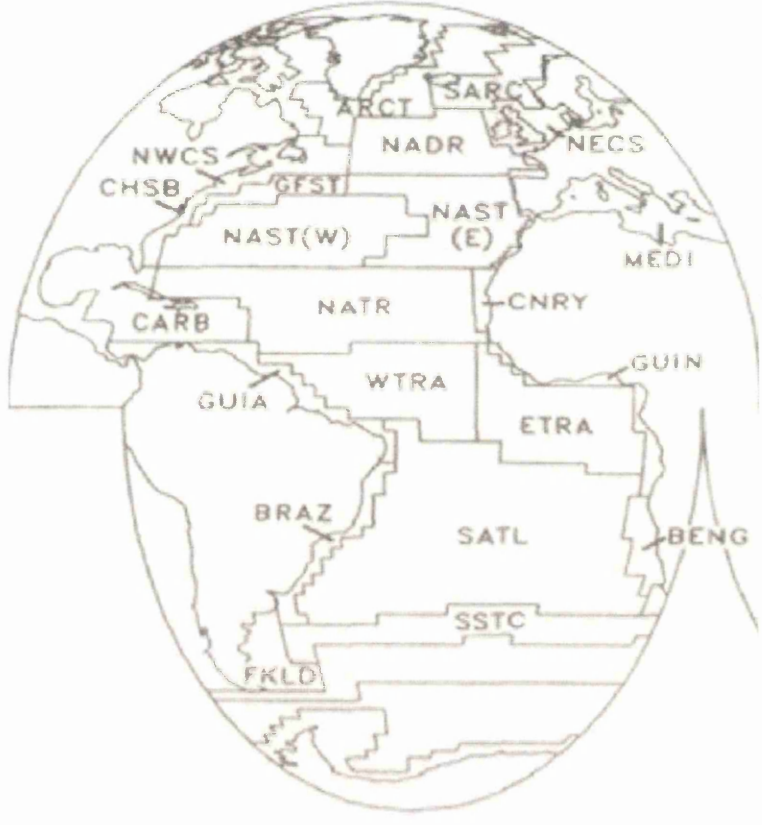


Figure 4.1. Biogeochemichal provinces in the Atlantic Ocean (modified from Longhurst et al., 1995) crossed during AMT-15. NECS-Northeast Atlantic Continental Shelf, NADR-North Atlantic Drift, NAST-North Atlantic Subtropical Gyre (West and East), CNRY-Canary Current Coastal, NATR-North Atlantic Tropical Gyre, WTRA-Western Tropical Atlantic, SATL-South Atlantic Tropical Gyre, SSTC-South Subtropical Convergence.

- NAST North Atlantic Subtropical Gyre (West and East). Comprises the central gyre of the North Atlantic polewards of the subtropical convergence. The mid- Atlantic Ridge constrains the main recirculation gyre within the western basin, and the biological properties of the eastern and western basin are such as to support Longhurst *et al.* (1995); Longhurst (2006) subdivision into two provinces.

- CNRY Canary Current Coastal. Comprises the seasonally varying regions of coastal upwelling from Cape Finisterre (43°N) to Cap Vert at 15°N, and extending seasonally to the Bissagos Islands at 11°N.

- NATR North Atlantic Tropical Gyre. Comprises the central gyre south of the subtropical convergence zone to the limit of westerly flow along the thermocline ridge at ≈10°N marking the northern edge of the North Equatorial Countercurrent (NECC).

- WTRA Western Tropical Atlantic. The region west of the mid-Atlantic Ridge and between the 10°N thermocline ridge and the subtropical convergence zone.

- SATL South Atlantic Tropical Gyre. Comprises the South Equatorial Current where it forms the equatorward limb of the central gyre.

- SSTC South Subtropical Convergence. The most northerly of the annular features of the Southern Ocean. The frontal zone is sufficiently dynamic to have an associated eddy field and includes several surface discontinuity fronts.

4.1. Materials and methods

AMT-15 was conducted on board the Royal Research Ship *Discovery* (cruise 284) from 17th September to 29th October 2004. RRS *Discovery* sailed from Southampton (UK), made a brief port call into Santa Cruz de Tenerife on 27th September (Canary Islands), surveyed the upwelling area in front of the Moroccan coast from 30th September to 3rd October, and arrived in Cape Town (South Africa) on 28th October (Fig. 4.2).

Along the transect, 72 stations were visited and 105 CTD deployments were made between 48.75°N and 40.0°S (Fig. 4.2).

Mesozooplankton samples were collected at 35 stations (Table 4.1, Fig. 4.2), always at night (together with pre-dawn CTD casts; between 02:00 to 03:00 GMT) in the upper 200 m of the water column, except at the shallow Stn 1 and Stn 26 (50 m) and at Stn 4 due to technical problems (125 m).

Quantitative mesozooplankton samples were collected by vertical hauls with a double WP-2 net (57 cm mouth diameter and 200 μm mesh) equipped with a filtering cod-end. Zooplankton samples were fixed with 4% buffered formaldehyde-seawater solution and analysed later in the laboratory under a dissecting stereoscope using a Bogorov chamber. At least 1/22 of the entire sample was analyzed taking repeated aliquots with a large mouth graduate syringe after accurate mixing (modified Stempel pipette method). *Clausocalanus* adults (females and males) were identified and counted at species level according to Frost and Fleminger (1968), while copepodid stages (mainly stage III-V, retained by the 200 μm mesh) were identified and counted at genus level.

Qualitative information on number of females carrying spermatophores or sac remains and number of spermatophores per female was also collected as an estimation of reproductive index.

Table 4.1. AMT-15 stations surveyed for mesozooplankton collection. Collection time is reported in Coordinated Universal Time (UTC), a high-precision atomic time standard.

Stn	COORDINATES		DATE	TIME	SONIC
	Latitude	Longitude	(dd/mm/yy)	(UTC)	DEPTH (m)
1	48°44.06 N	7°50.25 W	19/09/04	1:46	150
4	47°54.92 N	14°36.90 W	21/09/04	1:50	4599
6	45°59.08 N	18°23.73 W	22/09/04	2:40	4069
8	42°32.93 N	19°50.28 W	23/09/04	2:11	5258
10	38°53.85 N	20°21.07 W	24/09/04	2:10	4509
12	35°05.98 N	20°51.10 W	25/09/04	2:08	5197
14	31°15.59 N	20°42.96 W	26/09/04	2:10	4838
16	29°07.67 N	16°57.97 W	27/09/04	2:07	5176
18	23°33.26 N	19°59.34 W	29/09/04	2:06	3846
20	21°22.27 N	18°49.54 W	30/09/04	2:03	3071
23	21°41.13 N	17°50.39 W	01/10/04	2:04	1004
26	21°20.39 N	17°20.34 W	02/10/04	2:25	75
29	21°18.18 N	18°34.77 W	03/10/04	2:07	2737
30	17°49.84 N	20°52.60 W	04/10/04	2:00	3117
32	14°18.01 N	21°45.13 W	05/10/04	2:05	4303
34	10°59.91 N	22°30.50 W	06/10/04	2:05	5089
36	07°50.55 N	23°13.73 W	07/10/04	2:06	4541
38	04°45.35 N	23°55.46 W	08/10/04	2:05	4375
39	02°30.48 N	24°26.07 W	09/10/04	2:37	4066
41	00°03.91 N	24°58.96 W	10/10/04	2:15	4237
44	06°50.81 S	25°00.34 W	12/10/04	2:07	5601
46	10°25.09 S	24°59.79 W	13/10/04	2:09	5195
48	14°10.62 S	24°59.71 W	14/10/04	2:09	4866
50	17°57.17 S	24°59.99 W	15/10/04	2:07	5366
52	20°38.30 S	23°40.32 W	16/10/04	2:06	5429
54	21°55.39 S	20°58.93 W	17/10/04	2:05	5201
56	23°33.88 S	17°30.26 W	18/10/04	2:04	4102
58	25°14.04 S	13°55.42 W	19/10/04	2:10	3073
60	26°53.11 S	10°20.19 W	20/10/04	2:04	3793
62	28°35.04 S	06°34.36 W	21/10/04	2:08	4172
64	31°08.47 S	03°55.29 W	22/10/04	1:08	4452
66	34°30.87 S	01°22.75 W	23/10/04	1:12	4393
68	37°49.66 S	01°13.55 E	24/10/04	1:05	4983
70	40°00.07 S	05°00.80 E	25/10/04	1:06	5001
72	40°00.91 S	10°01.59 E	26/10/04	1:10	4898

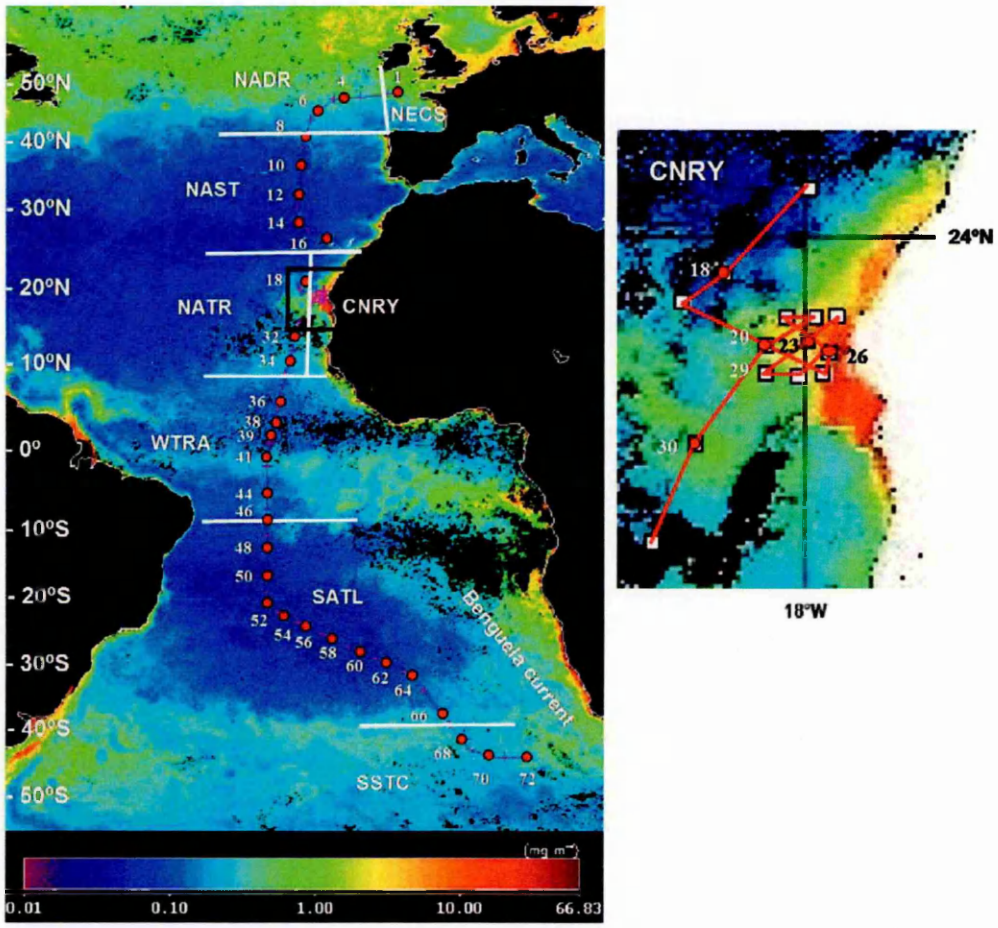


Figure 4.2. AMT-15 cruise track with the stations surveyed for mesozooplankton collection overlaid onto SeaWiFS chlorophyll composite for September 2004. Area surveyed at the North West African Upwelling enlarged. Biogeochemical provinces crossed during the cruise according to Longhurst *et al.* (1995) and Longhurst (2006): NECS Northeast Atlantic Continental Shelf; NADR North Atlantic Drift Province; NAST North Atlantic Subtropical Gyral Province; NATR North Atlantic Tropical Gyral Province; CNRY Canary Current Coastal Province; WTRA Western Tropical Atlantic Province; SATL South Atlantic Gyral Province; SSTC South Subtropical Convergence Province.

Environmental parameters were recorded in the upper 300 m using a Seabird 9/11+ CTD system with a SBE 32 Carousel fitted with 24 by 20 l Ocean Test Equipment (Niskin type) external spring water bottles. Environmental data were provided by the Natural Environment Research Council (NERC) personnel and are available at the British Oceanographic Data Center (BODC). Chl *a* concentrations measured at discrete depths along the transect (kindly provided by Dr. A. Poulton and Dr. P. Holligan and available at BODC) were also used together with CTD data to identify environmental provinces.

4.2. Results

Environmental parameters

Vertical profiles of temperature, salinity (Fig. 4.3a) and fluorescence as well as Chl *a* measurements in the water column (Fig. 4.3b), showed environmental gradients according to the different regions crossed during the AMT-15 cruise.

The first surveyed station (Stn 1) was in the Celtic Sea, on the European continental shelf, at ca. 275 km to the west of the western mouth of the English Channel near the shelf break (Fig. 4.2). The mixed layer at Stn 1 was in the upper 50-60 m, deeper at Stn 4 (in the upper 76-86 m), but from Stn 6, across the north Atlantic gyre and until the upwelling area (Stn 23) it maintained in the upper 40-60 m (Fig. 4.3a, 4.4a). At Stn 26, the mixed layer was shallower (upper 20-30 m) and upwelling water was about 2-3°C colder and >1 psu more saline than the nearest stations (Stn 23 and Stn 29). The mixed layer reappeared in the upper 50 m at Stn 29 (same coordinates as Stn 20) deepening southward until 140-150 m at Stn 46 located at the edge of the south Atlantic gyre (Fig. 4.3a, 4.4a). In the south Atlantic gyre, the mixed layer remained at this depth or even

deeper (160-170 m). From Stn 64 to the end of the cruise (Stn 72), the mixed layer was deeper than 200 m (Fig. 4.3a, 4.4a).

Temperature in the mixed layer was 16.6°C (±0.3) at Stn 1, decreased at Stn 4 (16.0°C; ±0.8), increasing again from Stn 6 (18.3±0.5°C) to Stn 14 (24.4±0.5°C). From Stn 14 to Stn 18, temperature in the mixed layer remained above 24°C. In the upwelling region, temperature of the water in the mixed layer decreased to around 23°C at Stn 20 and Stn 29 (same coordinates), to 20.9°(±0.7) at Stn 23 and 19.1°C (±0.8) at Stn 26 (Fig. 4.4b). At Stn 30, temperature in the mixed layer was 26.5°C (±0.4) increasing one degree per station, reaching the maximum value at Stn 34 (28.6±0.5°C). Temperature in the mixed layer remained above 26°C until the equator (Stn 41). After that, temperature in the mixed layer decreased gradually until it reached minimum values that were recorded along the transect (Stn 70: 10.0±0.7°C). It increased about 2 degrees at Stn 72 (Fig. 4.4b). Below the mixed layer, mean temperature recorded until 300 m (except at shallow stations) ranged from 8.5 to 18.3°C (Fig. 4.4b).

Salinity above the mixed layer showed a similar pattern as temperature at these stations before the upwelling region. It gradually increased from Stn 1 (35.4±0.01 psu) to Stn 14 (37.1±0.4 psu) where it reached a first peak value. It decreased slightly during the next three stations, showing a strong decrease in the upwelling region (Stn 20 to Stn 29). At Stn 26, water in the mixed layer was more saline than at the adjacent stations (Fig. 4.4c). From Stn 30 to Stn 44, salinity was below 36.5 psu, reaching its minimum value at Stn 38 (35.3±0.6 psu). After the equator (Stn 44) salinity rapidly increased with a peak at Stn 50 (37.1±0.1 psu). After that, salinity in the mixed layer decreased constantly until reaching the minimum recorded for this layer (Stn 70: 34.5±0.1 psu)(Fig. 4.4c). At Stn 72, a strong decrease in salinity was observed in the upper 10-12 m of the water column

(30.1 psu) (Fig. 4.3a). Salinity recorded below the mixed layer was equal to those recorded above the mixed layer at the cold stations (Stns 1, 4, 68-72), or slightly lower until the equator where the difference increased considerably in the center of the south Atlantic gyre (from 0.9 to 1.4 psu of difference)(Fig. 4.4c).

Chl *a* was concentrated in the mixed layer (50 m) at the first two stations (Stn 1: 0.53 ± 0.03 μg Chl *a* L^{-1}), then Chl *a* concentration decreased in the mixed layer away from the continental shelf and entering the north Atlantic gyre (from 0.2 μg Chl *a* L^{-1} at Stn 6 to 0.1 μg Chl *a* L^{-1} at Stn 16). A clear DCM appeared below the mixed layer at 50 m at Stn 6 (0.7 μg Chl *a* L^{-1}) then it deepened southward down to 115 m at Stn 16 (0.2 μg Chl *a* L^{-1}), rising again to 50 m at Stn 18 (0.5 μg Chl *a* L^{-1})(Fig. 4.3b and Fig. 4.4d). From Stn 20 to Stn 26, Chl *a* was concentrated in the upper 30 m of the water column, increasing from 0.6 μg Chl *a* L^{-1} (± 0.2) at Stn 20 to 6.1 ± 2.9 μg Chl *a* L^{-1} atStn 26, the shallowest station in the upwelling region (Fig. 4.3b and Fig. 4.4d). DCM reappeared at 45 m at Stn 29 where high Chl *a* concentration was measured (1.4 μg Chl *a* L^{-1}) (Fig. 4.3b). From Stn 30 to the equator (Stn 41), Chl *a* concentrations in the mixed layer ranged from 0.1 to 0.2 μg Chl *a* L^{-1}, while those measured at the DCM between 50-70 m ranged from 0.3 to 0.5 μg Chl *a* L^{-1} and were located (Fig. 4.3b, 4.4d). Once we had crossed the equator, Chl *a* concentration in the mixed layer ranged from 0.1 to 0.03 μg Chl *a* L^{-1}; in the DCM that was located below 100 m (down to 180 m at Stn 56 in the middle of the south Atlantic gyre), Chl *a* concentrations ranged from 0.2 to 0.3 μg Chl *a* L^{-1} (Fig. 4.3b, 4.4d). Chl *a* was homogeneously distributed in the water column (down to 80 m) from Stn 68 to Stn 72, ranging from 0.3 to 0.4 μg Chl *a* L^{-1} (Fig. 4.3b, 4.4d).

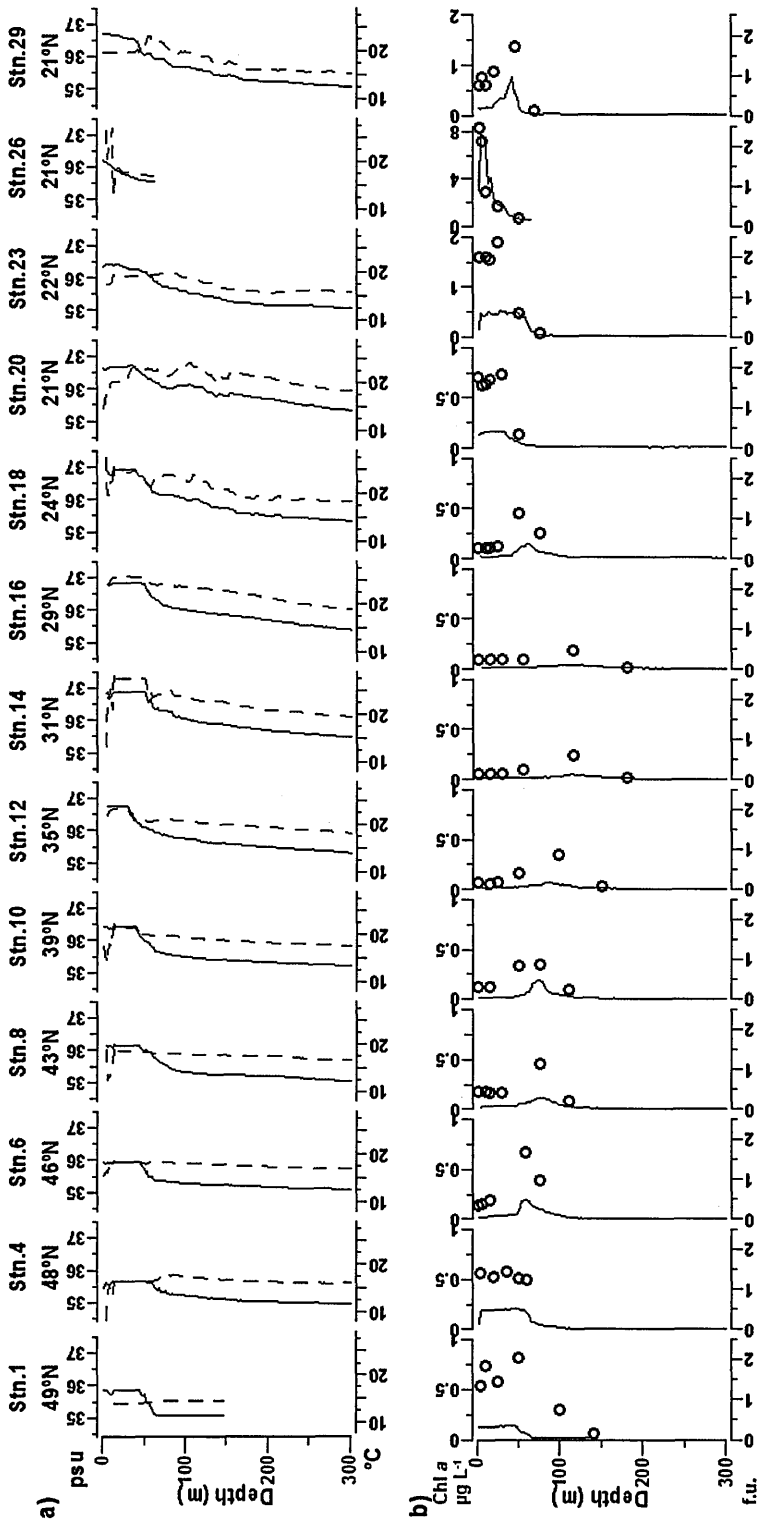


Figure 4.3. Vertical distribution of environmental parameters recorded by CTD in the upper 300 m during AMT-15 cruise. a) Temperature (°C; continuous line) and salinity (psu; discontinuous line); b) fluorescence profiles (f.u.) and chlorophyll *a* concentrations measured at discrete depths (μg Chl *a* L^{-1}).

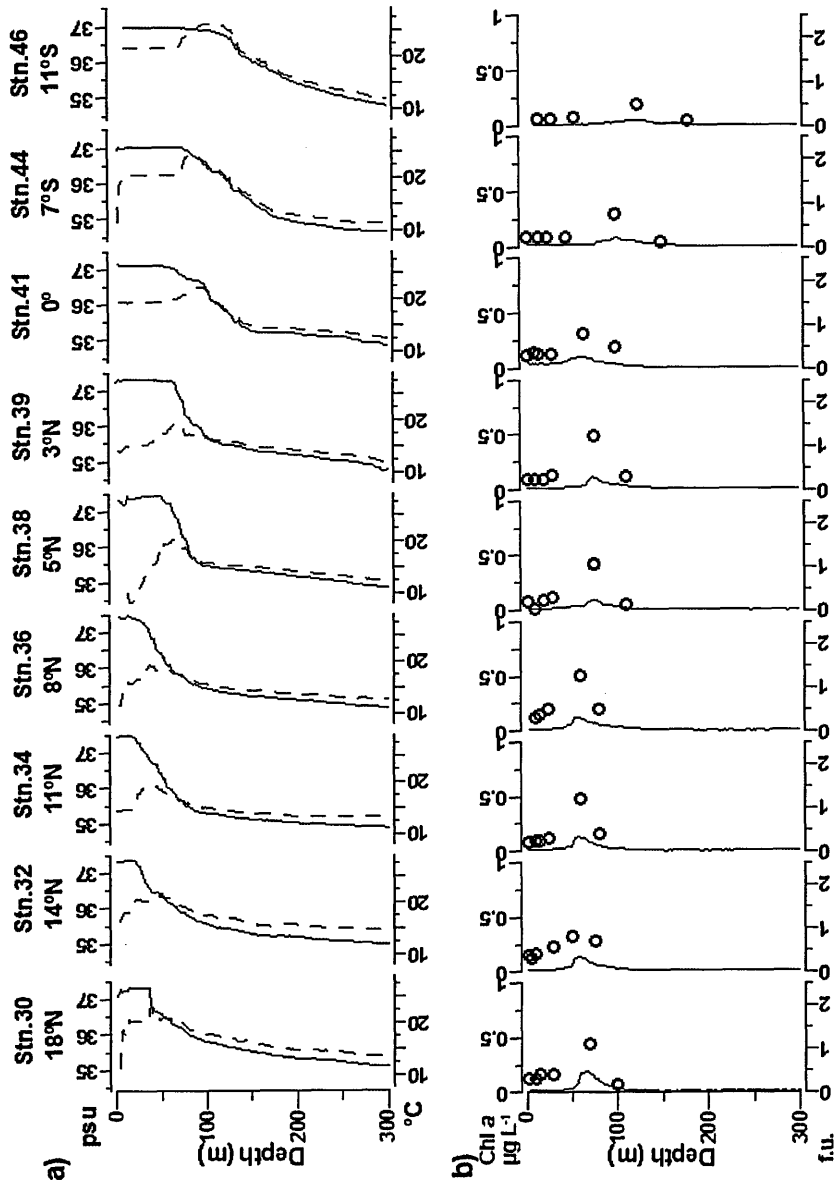
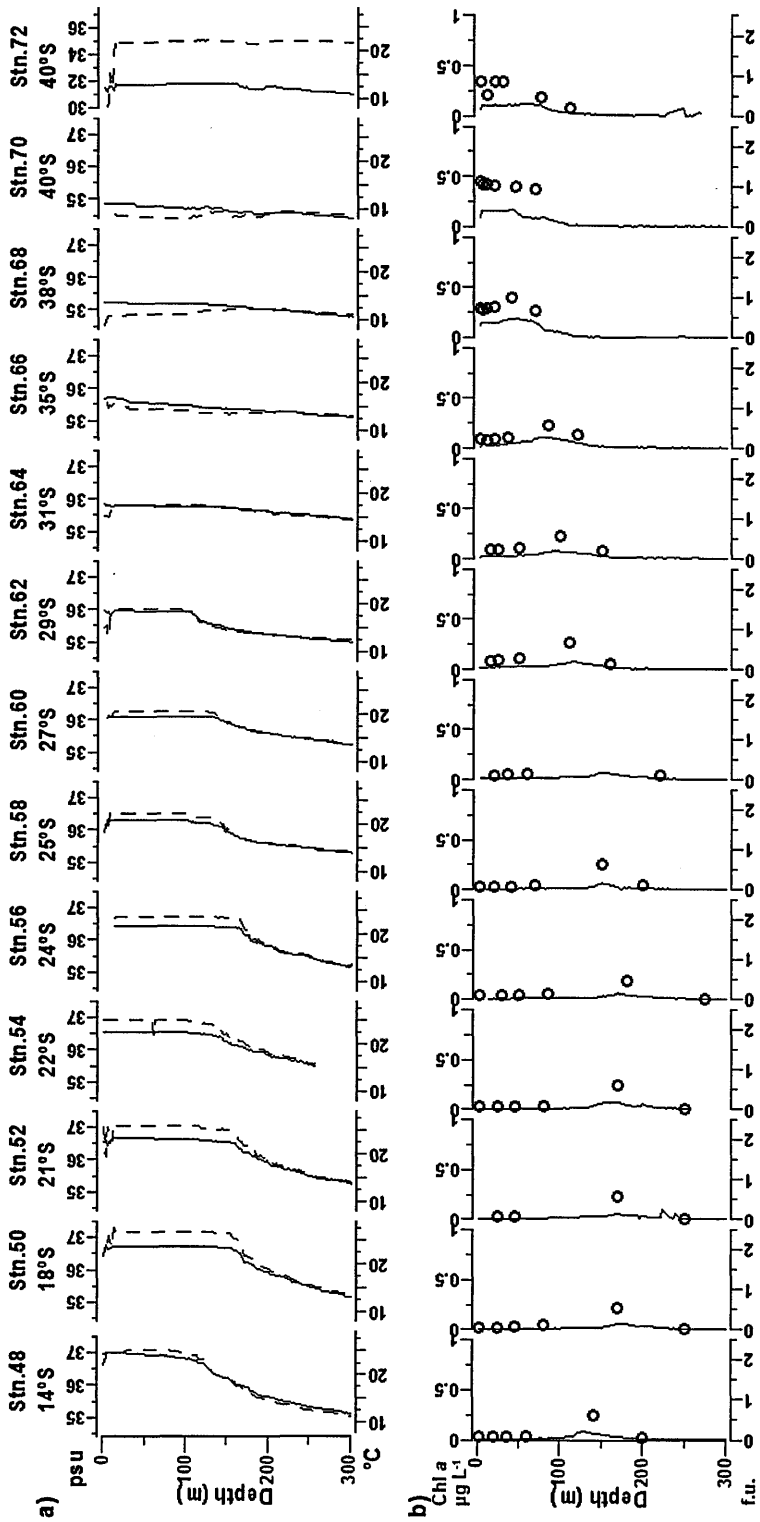


Figure 4.3 (Continued)



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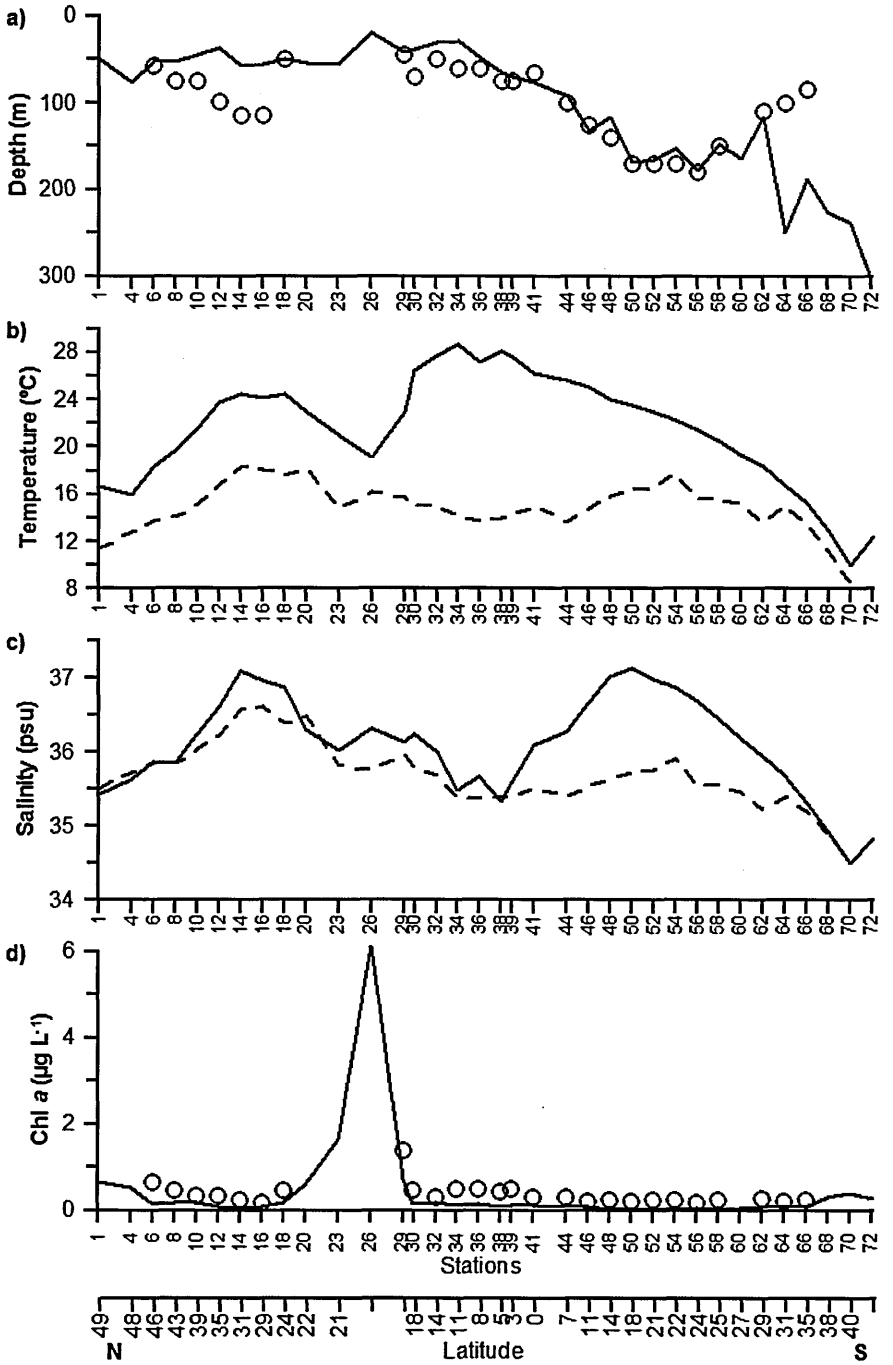

Figure 4.4. (a) Mixed layer depth (calculated according to the temperature criterion, it is the depth at which the temperature change from the surface temperature is 0.5°C; continuous line) and deep chlorophyll maximum depth (open circles) recorded along AMT-15 cruise. (b) Mean values of recorded temperature in the mixed layer (continuous line) and below the mixed layer (discontinuous line). (c) Mean values of recorded salinity in the mixed layer (continuous line) and below the mixed layer (discontinuous line). (d) Chl *a* concentration measured in the mixed layer (line) and at the DCM (open circles).

Clausocalanus

Clausocalanus was more abundant in the Northern hemisphere, showing the major peak at Stn 8 (360.4 ind. m^{-3})(Fig. 4.5a). Its abundance decreased tenfold in the northern gyre (Stns 10-16; 38.1 ± 22.8 ind. m^{-3}). *Clausocalanus* abundance showed a second peak South of the Canary Islands (Stn 18, 186.5 ind. m^{-3}), it increased through the upwelling area (Stn 26, 116.3 ind. m^{-3}), and showed a third peak outside the upwelling area (Stn 30, 257.2 ind. m^{-3}), then it decreased fourfold on average at the next five stations (Stns 32-39, 64.6 ± 24.4 ind. m^{-3}). At the equator, *Clausocalanus* showed a fourth peak in abundance (Stn 41, 163.9 ind. m^{-3}). At the following two stations, its abundance was similar to that recorded at the five stations surveyed north the equator. In the south Atlantic gyre (Stns 48-66), *Clausocalanus* abundance reached its minimum values (25.5 ± 9.5 ind. m^{-3}). A sudden increase of the genus abundance occurred at Stn 68 and Stn 70 (106.1 ± 8.9ind. m^{-3}) before decreasing at the last station (Stn 72, 27.7 ind. m^{-3}).

The genus *Clausocalanus* represented on average 22.7% (± 11.2) of total copepod abundances along the transect. Its contribution was lower at coastal stations (7.6% at Stn 1 and 9.4% Stn 26) and was maximum in the north (up to 57% at Stn 8)(Fig. 4.5b). Peaks of *Clausocalanus* relative contribution were in accordance with abundance peaks. The genus relative contribution described a Gaussian bell shape around the peaks, except at the equator. Despite the fact that *Clausocalanus* abundance in the southern gyre was the lowest observed along the transect, its relative contribution from the equator (Stn 41) to the southernmost station but one (Stn 70) was on average 23.4% (± 6.0). Genus contribution at the southern station was low (Stn 72; 10.4%) as at the coastal stations (Stn 1 and Stn 26).

Clausocalanus population mainly consisted of adult females (49.8 ± 12.0%)

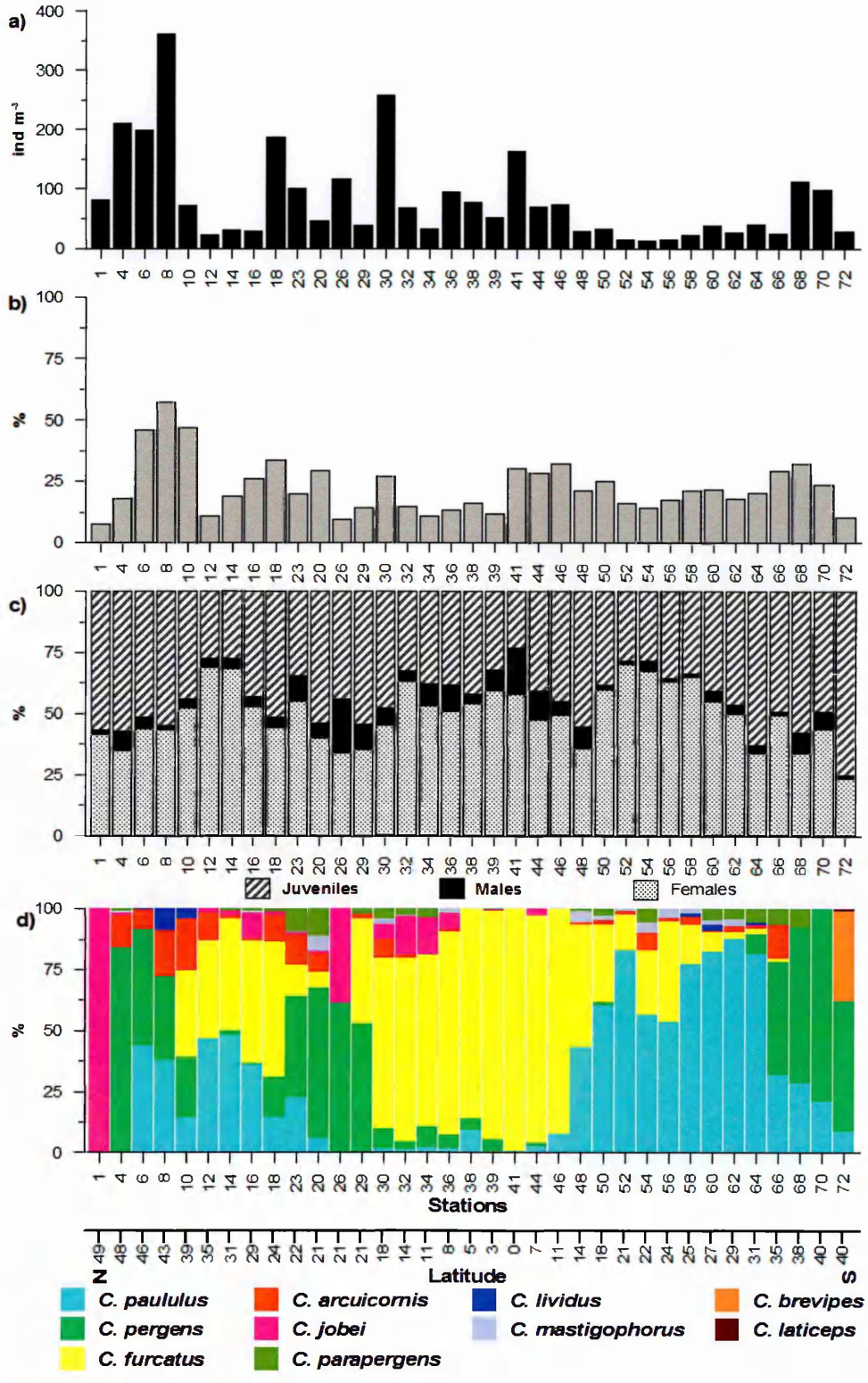

Figure 4.5. AMT-15 cruise: (a) *Clausocalanus* abundance; (b) *Clausocalanus* relative contribution on total copepods; (c) *Clausocalanus* population structure; (d) *Clausocalanus* species composition (adult males and females). Data is reported for each sampled station and its latitude is indicated in axis at the bottom of the figure.

and copepodids (44.0 ± 11.7%). Females predominated in the gyres and subtropical stations (Stn 32-46) reaching up to 70.2% of relative contribution at Stn 52 (Fig. 4.5c), while copepodids predominated in the northern and southern part of the transect and at Stn 48, reaching their maximum contribution at the last station (Stn 72, 75.1%). Males represented on average 11.2% (± 8.2) of *Clausocalanus* adult populations. They accounted for up to 39.8% at Stn 26 (in the upwelling area) and 24.6% at the equator (Stn 41), reaching a minimum contribution in the South Atlantic gyre (2.0%).

Eleven *Clausocalanus* species were found along the transect: eight had a wide range of latitudinal distribution (*C. paululus*, *C. pergens*, *C. furcatus*, *C. arcuicornis*, *C. jobei*, *C. parapergens*, *C. lividus*, and *C. mastigophorus*) while three species were observed only in the south Atlantic (*C. ingens*, *C. brevipes*, and *C. laticeps*). Three species were the most representative along the transect and accounted for 85.1% of total adult (females and males) *Clausocalanus* (Fig. 4.5d): *C. furcatus* 35.7% (± 34.5), *C. paululus* 28.9% (± 29.2) and *C. pergens* 20.5% (± 26.8). Less common species were *C. jobei* (5.8 ± 18.0%), *C. arcuicornis* (4.3 ± 5.9%), *C. parapergens* (2.2 ± 3.0%), *C. mastigophorus* (1.0 ± 1.6%), *C. lividus* (0.5 ± 1.7%), *C. brevipes* (1.0 ± 6.2%). *C. laticeps* and *C. ingens* were very rare (0.03 and 0.01%, respectively).

C. furcatus relative contribution reached up to 99.3% at the equator (Stn 41; Fig. 4.5d) and it was the dominant species from Stn 30 to Stn 46. *C. paululus* represented 37.6% (± 12.4) of *Clausocalanus* species from Stn 6 to Stn 16. It was the most representative species in the south Atlantic gyre (Stns 50-64, 72.8 ± 13.7%) accounting up to 87.5% of *Clausocalanus* species at Stn 60. *C. pergens* was mostly represented in three regions: the northern non continental stations (Stns 4-8), the upwelling area (Stns 20-29) and the southern stations (Stn 66-72). *C. jobei* was

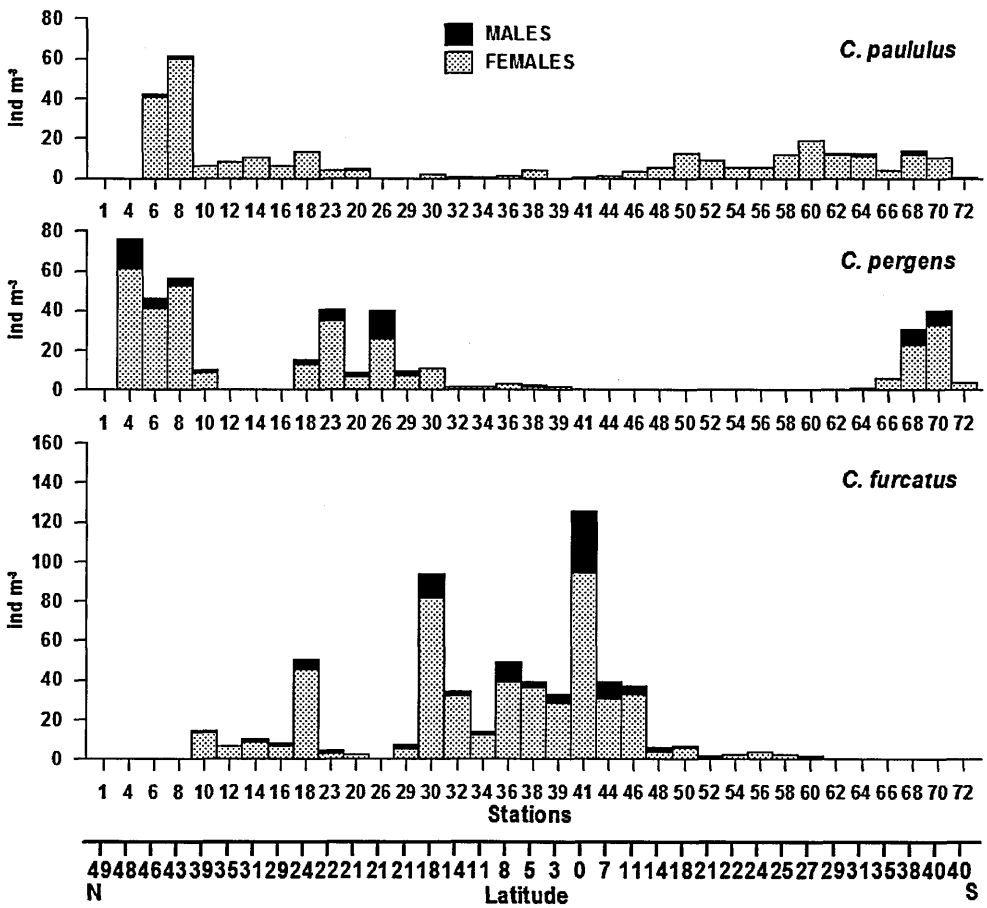

Figure 4.6. (a) Abundance of *C. paululus*, *C. pergens* and *C. furcatus* (females in grey and males in black) along the AMT-15 transect.

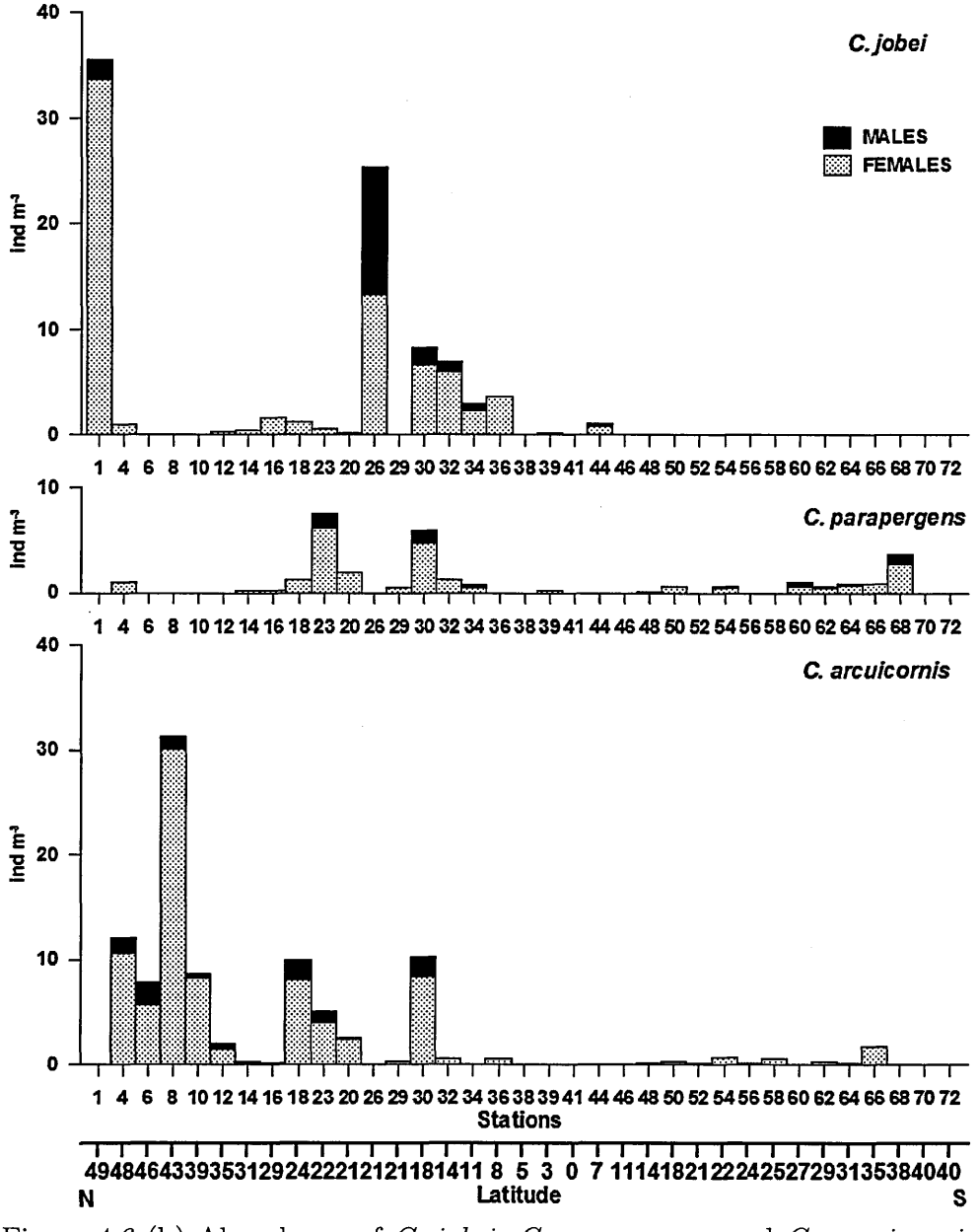

Figure 4.6 (b) Abundance of *C. jobei*, *C. parapergens* and *C. arcuicornis* (females in grey and males in black) along the AMT-15 transect.

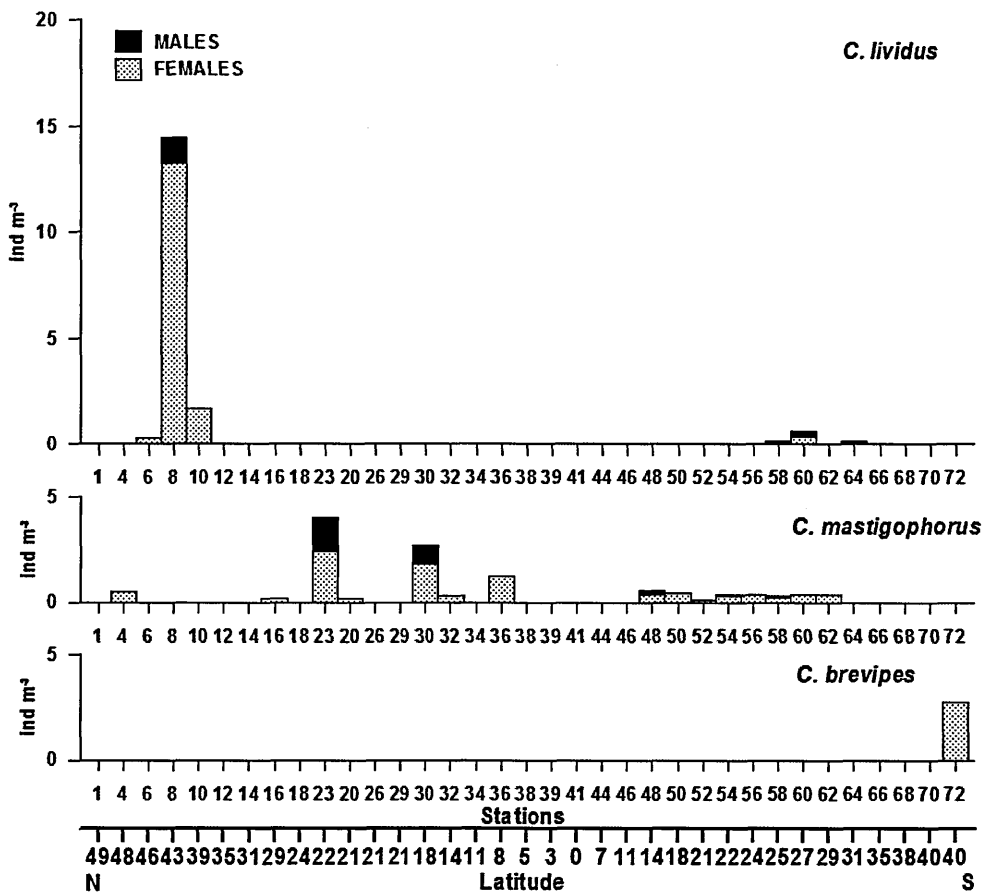

Figure 4.6 (c) Abundance of *C. lividus*, *C. mastigophorus* and *C. brevipes* (females in grey and males in black) along the AMT-15 transect.

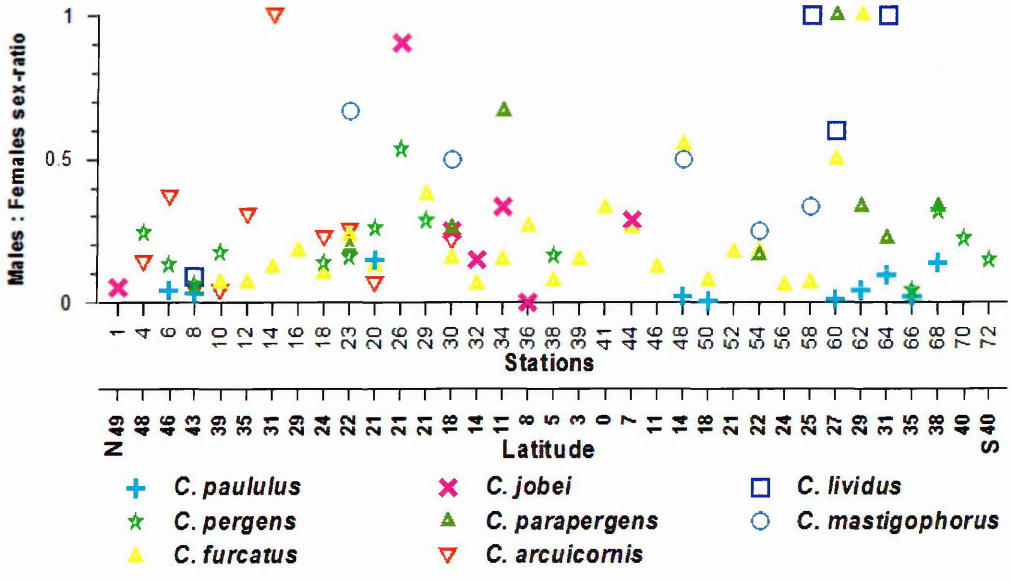

Figure 4.7. AMT-15 cruise. *Clausocalanus* species M:F sex-ratio.

the unique species occurring on the continental shelf (Stn 1), and represented 38.9% at Stn 26 (where the remaining 61.1% was represented by *C. pergens*) also located on the continental shelf. No other species among the least representative ones reached more than 20% of relative contribution at any station, except *C. brevipes* which represented 36.6% of *Clausocalanus* species at Stn 72, the only station where it was found (Fig. 4.5d).

C. paululus was the third most abundant species along the transect (mean abundance 8.2 ± 12.2ind. m^{-3})(Fig. 4.6a). It was absent at the two northernmost stations (Stn 1 and Stn 4), showing its highest abundance in the north Atlantic drift (Stn 6 and Stn 8, mean abundance 51.7 ind. m^{-3}, ± 13.5). Then, it suddenly decreased to 4.9 ind. m^{-3} (± 3.9) over the rest of the north Atlantic being absent in the upwelling area (Stn 26 and Stn 29) and near the equator (Stn 39) while it was the most abundant species in the south Atlantic gyre (from Stn 48 to Stn 64 its abundance was 10.1 ind. m^{-3}, ± 4.6). Males were most abundant in the north

Atlantic (at Stn 6 and Stn 8, 1.8 ind. m^{-3} at each station). This species showed the lowest M:F sex-ratio (maximum at Stn 20, 0.1)(Fig. 4.7).

C. pergens was the second most abundant species (11.6 ± 19.4 ind. m^{-3} in mean abundance)(Fig. 4.6a). This species was more abundant in three regions: in the north Atlantic drift (Stns 4-8, 59.5 ± 14.9 ind. m^{-3}), in the upwelling region (Stn 23 and 26, 40.2 ± 0.6 ind. m^{-3}; being the most abundant species at Stn 26 where only two *Clausocalanus* species occurred) and at the southernmost stations of the transect (Stn 68 and 70, 35.3 ± 6.7 ind. m^{-3}). This species was absent in the north Atlantic drift (Stn 1 and Stn 12) and in the south Atlantic gyre (Stn 46-62). Males were maximum at the north Atlantic drift and at the coastal upwelling station (Stn 4 and 26, 14.9 and 13.9 ind. m^{-3}, respectively), showing at the last site the highest M:F sex-ratio (0.5)(Fig. 4.7).

C. furcatus was the most abundant species along the transect (mean abundance 16.9 ± 28.1 ind. m^{-3}) reaching the highest abundance at the equator (Stn 41, 125.4 ind. m^{-3})(Fig. 4.6a). This species was mostly concentrated in the tropical region (from Stn 30 to Stn 46) and was absent only at the coastal stations (Stn 1 and 26) and at the southernmost stations (Stn 68-72). Male abundance was highest at the equator (Stn 41; 31.0 ind. m^{-3}) while the highest M:F sex-ratio was observed in the south Atlantic gyre (Stn 62, 1.0)(Fig. 4.7).

Among the least abundant species, *C. jobei* and *C. arcuicornis* presented similar mean abundance along the transect (2.7 ± 6.1ind. m^{-3}, and 2.6 ± 7.3 ind. m^{-3}, respectively) but had different distributions (Fig. 4.6b). *C. jobei* showed its peak abundance at the northernmost station on the continental shelf (Stn 1, 35.6 ind. m^{-3}, the only species that occurred at this station) and at the coastal station in the upwelling area (Stn 26, 25.3 ind. m^{-3} the second most abundant species of the two that occurred at this station). In the northern part of the

transect, *C. jobei* almost disappeared after its peak abundance (0.7 ind. m^{-3} at the north of the upwelling and 3.8 ind. m^{-3} after the upwelling). At the southern part of the transect, *C. jobei* was observed at one station at very low abundance (Stn 64, 0.08 ind. m^{-3}). Maximum *C. jobei* male abundance and maximum M:F sex ratio were recorded at the upwelling coastal station (Stn 26, 12.0 ind. m^{-3} and 0.9, respectively)(Fig. 4.7).

C. arcuicornis showed its peak abundance in the north Atlantic (Stns 4-10, maximum abundance 31.3 ind. m^{-3} at Stn 8; Fig. 4.6b). In the northern part of the transect, *C. arcuicornis* showed a strong population decline after Stn 10, increasing again at the boundary stations near the upwelling region (Stns 18 and 30). At the southern part of the transect, *C. arcuicornis* was present in the southern Atlantic gyre (mean abundance 0.4 ± 0.5 ind. m^{-3}). Maximum *C. arcuicornis* male abundance was recorded in the north Atlantic drift (Stn 6, 2.1 ind. m^{-3}) as well as the maximum M:F sex-ratio values (Stn 14, 1.0)(Fig. 4.7).

C. parapergens (mean abundance 0.8 ± 1.6 ind. m^{-3}) showed two peaks in abundance: the first in the upwelling area (Stn 23) and a second one in the tropical Atlantic region (Stn 30) (7.4 and 5.9 ind. m^{-3}, respectively)(Fig. 4.6b). It was present at the beginning and at the end of the south Atlantic gyre at low abundance where it showed a third small peak abundance at Stn 68 (3.64 ind. m^{-3}). *C. parapergens* male maxima were observed in accordance with population peaks (Stn 23 and 30, 1.2 ind. m^{-3}, each) while the maximum M:F sex-ratio was observed in the south Atlantic gyre (Stn 60, 1.0)(Fig. 4.7).

C. lividus (mean abundance 0.5 ± 2.4 ind. m^{-3}) was present at six stations: at three in the north (Stns 6-10) where abundance was highest (Stn 8, 14.5 ind. m^{-3}), and at three stations in the south (Stns 58-64 but 62) where it was scarce (0.3 ± 0.3 ind. m^{-3})(Fig. 4.6c). *C. lividus* males maximum occurred in the north

Atlantic gyre (Stn 8, 1.2ind. m^{-3}) while the maximum M:F sex-ratio was observed in the south Atlantic gyre (Stn 58, 1.0)(Fig. 4.7).

C. mastigophorus (mean abundance 0.3 ± 0.8 ind. m^{-3}) had its peak abundance at the same stations as *C. parapergens* (Stns 23 and 30, 4.0 and 2.7 ind. m^{-3}, respectively)(Fig. 4.6c). It was present in the south Atlantic gyre where it was very scarce (Stns 48-62, 0.4 ± 0.1 ind. m^{-3}). Male were most abundant at the population peak (Stns 23 and 30, 1.6 ind. m^{-3} and 0.9 ind. m^{-3}, respectively) where at the first station the maximum M:F sex-ratio was observed (0.7).

C. brevipes and *C. laticeps* were only present at Stn 72 at low abundance (2.8 and 0.1 ind. m^{-3}, respectively) while *C. ingens* was only observed at Stn 70 at very low abundance (<0.1 ind. m^{-3}). No males were observed in any of these three species.

The cluster analyses showed that physical (temperature and salinity) and biological (chlorophyll concentration and deep chlorophyll maximum depth) parameters in the mixed layer during the cruise fit with the biogeochemical provinces described on previous AMT cruises whose tracks overlapped with AMT-15 (mainly in the northern hemisphere Hooker *et al.*, 2000) and with those described at global scale (Longhurst *et al.*, 1995; Longhurst, 2006) despite using data from the mixed-layer rather than from the surface (Fig. 4.8, 4.9). Four main clusters were revealed. Latitude played a marked role to further subdivision into provinces. The observed differentiation among clusters and intra-clusters was due both to local circulation features and structure of *Clausocalanus* assemblages, and mostly reproduced the separation among biogeochemical provinces (here presented from north to south):

- North European Coastal province (NEC)(Stn 1, Cluster III) showed similar environmental features as the next four stations (Stn 4–10), but Stn 1 was located on the edge of the European continental shelf. Despite the fact that

it was characterized by North Atlantic Drift water (NADRw) that advected into the area (as was observed during AMT–1; Hooker et al. 2000), it was not exactly the North Atlantic Drift (NADR) province having completely different *Clausocalanus* species. *C. jobei* was the only species occurring at this station. This species did not occur again until near the Canary Islands but only at high concentrations at the coastal station in the upwelling system. The presence of this coastal species indicates that Stn 1, still belonged to the North European Coastal province (NEC Longhurst *et al.*, 1995; Longhurst, 2006).

- North Atlantic Drift province (NADR) (Stns 4-10, Cluster III) is affected by high wind speed in autumn and winter, deepening the mixed layer through the seasons. During AMT–15, we were affected by the remains of the Hurricane Ivan that generated heavy sea (force 9 waves) in the area. Mixed layer at stations occupied by the NADRw were characterized by low temperatures (18.4 ± 2.3°C), mid salinity (35.8 ± 0.3 psu) and high Chl *a* concentrations (0.34 ± 0.24 μg Chl *a* L^{-1}) with the occurrence of a DCM at 40–75 m, lying progressively deeper to the south. The highest adult *Clausocalanus* abundance on the whole transect was recorded in this province. At species level, *C. paululus* and *C. pergens* had their peak abundance in this province (>50 ind. m^{-3}). While *C. paululus* was absent at the northernmost station of the province and concentrated in the center, *C. pergens* abundance decreased to the south where both species had low abundance (5-10 ind. m^{-3}). There, *C. furcatus* appeared (15 ind. m^{-3}). Species *C. arcuicornis* and *C. lividus* were also present, showing their peak abundance at Stn 8 (30 and 15 ind. m^{-3}, respectively). *C. parapergens* and *C. mastigophorus* were scarce (<1 ind. m^{-3}), occurring only at the northernmost station (Stn 4).

- North Atlantic Subtropical Gyre (East) province (NAST–E) (Stns 12-16, Cluster I). Cold fronts in autumn erode the seasonal thermocline in this area. Temperature in the mixed layer was 24.1°C ($\pm$0.3), salinity was maximum (36.9 $\pm$ 0.2 psu) and Chl *a* concentration was very low (0.08 $\pm$ 0.02 μg Chl *a* L^{-1}). The DCM occurred at 90–120 m. Adult *Clausocalanus* abundance in this province was very low (<20 ind. m^{-3}), the most abundant species were *C. paululus* and *C. furcatus* (5-10 ind. m^{-3}, each one) while *C. pergens*, *C. arcuicornis*, *C. parapergens*, *C. jobei* and *C. mastigophorus* were scarce (<2 ind. m^{-3}) and *C. lividus* was absent.

- Canary Current Upwelling province (CCUp)(Stn 18-29, Cluster II; except Stn 26, Cluster IV) is located south of the Canary Islands. This area is characterized by cool filaments that exhibit strong vorticity and that are developed from persistent upwelling cells and may extend far beyond the shelf edge (van Camp et al. 1991). Stn 26 (21.3°N) was the only coastal station in this province. It was in an upwelling filament where the coldest upwelled water was recorded in the area. Sea-surface temperature and atmospheric temperature were so different that we were surrounded by a thick fog. The highest Chl *a* concentrations were measured (6.1μg Chl *a* L^{-1} in the mixed layer) and the sea was brown viewed from the surface. The northern station of the province was the less eutrophic and was dominated by *C. furcatus* (50 ind. m^{-3}). *C. paululus*, *C. pergens* and *C. arcuicornis* occurred at low abundance (10-15 ind. m^{-3}). The other four stations lined in a longitudinal transect (19°-17°W) at 21°N. The westernmost stations were less abundant in *Clausocalanus* species than the easternmost (20 vs 65 ind. m^{-3}). No specific dominance was observed in the westernmost stations while *C. pergens* predominated at the easternmost station (40 ind. m^{-3})

and *C. jobei* and *C. pergens* at the coastal station (25 ind. m^{-3}).

- North Atlantic Tropical and Western Tropical Atlantic provinces (NATR-WTRA) (Stn 30-41, Cluster II). This area includes two major circulation structures: the North Equatorial Current (NEC) and the North Equatorial Counter Current(NECC). The NEC is the continuation of the Canary Current that at approximately 12–14°N turns westward where it becomes the southern boundary of the North Atlantic Gyre (NAG), as well as the southern boundary of the NATR province. According to the latitude, only Stn 30 and Stn 32 (located betwen Cape Verde Islands and the Africa) would be included in the NATR province. The second circulation structure is the NECC that flows easterly and it is highly seasonal being strongest in the boreal autumn (from August to October). This current is known to transport Amazon water eastward (Dessier and Donguy, 1994). A core of Amazon water was seen at Stn 38 (4.8°N), evident as less saline warm water. Stations surveyed in the NWTRA region showed a progressive deepening of the mixed layer about 40–100 m, the highest recorded temperatures along the transect (27.4 ± 0.9°C), similar salinity as the NADR province (35.8 ± 0.3 psu) and low Chl *a* concentrations (0.13 ± 0.03 μg Chl *a* L^{-1}). The DCM remained at about 50-70 m depth all along the province. Adult *Clausocalanus* abundance was similar to that recorded at the offshore stations in the CCUp province (100 ind. m^{-3}), but at the species level *C. furcatus* was the dominant species (15-125 ind. m^{-3}). *C. pergens*, *C. arcuicornis*, *C. jobei* and *C. parapergens* were present in low abundance (5-10 ind. m^{-3}) at the northern stations and rapidly decreased or even disappeared towards the south. *C. mastigophorus* was present but scarce at the northern stations (3 ind. m^{-3}) and disappeared towards the south. *C. lividus* too was absent in this

province.

- South Atlantic Gyre province (SAG)(Stns 44-60, Cluster I). The mixed layer temperature and salinity in this province were similar to these recorded in the NATR province (22.8 ± 2.1°C, 36.7 ± 0.3 psu). Chl *a* concentrations were the lowest recorded along the AMT–15 (0.05 ± 0.02 μg Chl *a* L^{-1}). The DCM decreased in depth up to 180 m at Stn 52. Adult *Clausocalanus* abundance was higher at the first two stations (Stn 44 and Stn 46; 40 ind. m^{-3}) while at the rest of the stations was between 10–20 ind. m^{-3}, recording the lowest values in the center of the gyre (Stns 54-56). *C. furcatus* dominated Stns 44-46 (35-40 ind. m^{-3}) but it suddenly decreased to low abundance at Stn 48 (5 ind. m^{-3}) and became scarce at the southernmost station of the province. *C. paululus* was co-dominant with *C. furcatus* at Stn 48 and in the center of the gyre (Stn 54-56) (5 ind. m^{-3}), then it decreased through the center of the gyre and increased again to the south. Other species were scarce and sporadic, and *C. lividus* reappeared at Stn 58. The markedly different species abundance and composition at the first two stations of this province (Stns 44, 46) and the fact that they showed less saline mixed layer (<37 psu) than the rest of stations, suggests that they were probably boundary stations with the clearly less salty WTRA province (due to the effect of Amazon waters) and the salty South Atlantic Gyre.

- Stns 62-66 (Cluster 3) were located in the southern part of the SAG province, under the 28°S. They were in a boundary area between the warmer preceding stations and the colder successive stations. The upper 200 m of the water column were mixed in this region and the DCM rose up from 120 m to 80 m. Temperature and salinity recorded here were lower than these recorded in previous stations of the provinces (16.8 ± 1.5°C, 35.6 ± 0.3 psu)

and Chl *a* concentrations were slightly higher than these recorded at the preceding cluster of stations (0.08 ± 0.01 μg Chl *a* L^{-1}). *C. paululus* was still the dominant species at the first two stations (15 ind. m^{-3}) becoming co-dominant with *C. pergens* at Stn 66 (5 ind. m^{-3}) while all the other species remained scarce (<2 ind. m^{-3}). *C. ingens* was observed in this region but at low concentrations (<0.1 ind. m^{-3}).

- South Subtropical Atlantic Convergence zone (SSTC)(Stns 68-72, Cluster III) were the southernmost stations and it is a region affected by the cold Subtropical Front (STF). The water column was completely mixed in this area and temperature and salinity were the lowest recorded along the transect (11.8 ± 1.6°C, 34.7 ± 0.2 psu) while Chl *a* concentration was similar to that recorded in the NADR (0.3 ± 0.07 μg Chl *a* L^{-1}). The influence of the sub-Antarctic water was noticed at Stn 72 where less salty cold water was recorded in the first upper 15 m of the water column. The DCM was observed above the 75 m depth in this area. Adult *Clausocalanus* abundance was high at the first two stations (50 ind. m^{-3}) and sharply decreasing at the southernmost station (Stn 72) where abundance was low (<10 ind. m^{-3}). *C. pergens* was the most abundant species (30–40 ind. m^{-3}), followed by *C. paululus* (10–15 ind. m^{-3}) at the first two stations. At the southern station, *C. brevipes* and *C. laticeps* appeared but in low numbers (3 and 0.1 ind. m^{-3}, respectively).

4.3. Discussion

Since the seminal work conducted by Frost and Fleminger in 1968, *Clausocalanus* species have never been analysed in a synoptic investigation over a large

latitudinal range and the fragmented information available till now mainly refer to coastal rather than open ocean areas.

In previous AMT cruises, the genus *Clausocalanus* was pooled together with *Paracalanus* and *Pseudocalanus* in the group "small calanoids" (Woodd-Walker, 2001). During AMT-15 in September-October 2004, *Clausocalanus* occurred through the whole surveyed latitudinal range (48.7°N- 40.0°S). It contributed least in coastal waters, and followed the same trend as "small calanoids" reported by Woodd-Walker (2001). Previous sparse records describe *Clausocalanus* as a dominant genus in the North Atlantic Ocean, while records at the southern hemisphere were mainly concentrated along the Brazil coast, off the Argentine coast and in the Sub-Tropical Convegence Zone (>40°S)(Table 4.2, 4.3).

Despite the large co-occurrence of *Clausocalanus* species in the upper 200 m of the Atlantic Ocean during AMT-15, species contribution showed a marked degree of latitudinal partitioning. Regarding species occurrence, *Clausocalanus* species biogeography in the Atlantic Ocean is here updated since Frost and Fleminger (1968) (Fig. 4.10):

- *C. paululus* was reported as a subtropical, circumglobal species (Frost and Fleminger, 1968) with antitropical distribution (i.e., it occurs at comparable latitudes across the equator but not in the tropics) in Pacific Ocean. During AMT-15, this species was continuously recorded from 46°N to 40°S with no apparent antitropical distribution. Frost (1969) reported the northernmost record of this species at 50°N, while the southernmost limit of occurrence was extended to 40°S during the AMT-15.

- *C. pergens* was reported by Frost and Fleminger (1968) as a warm temperate, circumglobal species, with an apparently biantitropical distribution in the Pacific Ocean. Williams and Wallace (1975) recorded this species

widely distributed in the North Atlantic, within and south-east of the north Atlantic drift system, reaching as far north as 59.5°N, while the southernmost limit was 35°S in the Atlantic Ocean and 45°S in the Indian Ocean. During AMT-15, this species was apparently more related to cold temperate waters, occurring from 47.9°N to 40°S in the Atlantic Ocean, with no apparent antitropical distribution in this Ocean.

- *C. furcatus* was reported as a tropical-subtropical, circumglobal species (Frost and Fleminger, 1968). Williams and Wallace (1975) recorded this species only in the western Atlantic to the south-east of the Grand Banks around 43°N, at the edge of the Gulf Stream System where Frost and Fleminger (1968) recorded its northern limit. The southern limit of this species recorded by Frost and Fleminger (1968) was 35°S. During AMT-15, *C. furcatus* was recorded from 38.9°N to 34.5°S, within the already recorded latitudinal range.

- *C. jobei* was reported by Frost and Fleminger (1968) as a tropical or tropical-subtropical, circumglobal species. According to these authors, *C. jobei* appeared to have a broadly neritic distribution throughout its range. This was also observed during AMT-15 since *C. jobei* occurred on the European continental shelf and in the Moroccan upwelling region. Its northernmost limit was recorded by Williams and Wallace (1975) at 52.5°N and its southernmost limit was recorded by Frost and Fleminger (1968) at 35°S in the Atlantic Ocean and 40°S in the Pacific Ocean. During AMT-15 this species was observed at 48.7°N up to 31.1°S, more abundant at coastal sites and only sporadically at offshore sites.

- *C. parapergens* was reported as a tropical-subtropical and circumglobal

species, despite its sporadic occurrence in the tropical Pacific (Frost and Fleminger, 1968). Williams and Wallace (1975) recorded it as far north as 47.5°N, while Frost and Fleminger (1968) recorded it as far south as 35°S in the Atlantic Ocean and 45°S in the Pacific Ocean. During AMT-15, *C. parapergens* was sporadically recorded from 47.9°N to 37.8°S.

- *C. arcuicornis* was reported as a tropical-subtropical, circumglobal species (Frost and Fleminger, 1968). Williams and Wallace (1975) recorded the northernmost occurrence of this species at 59.5°N, while the southernmost occurrence was reported by Frost and Fleminger (1968) at 35°S in the Atlantic Ocean and 40°S in the Pacific Ocean. During AMT-15, this species was recorded form 48°N to 34.5°S, being absent from 4.8°N to 14.2°S suggesting that this species might be antitropical in the Atlantic Ocean.

- *C. brevipes* was reported as a subantarctic, circumglobal species occurring at 40°-60°S (Frost and Fleminger, 1968). This species was recorded during AMT-15 at the last surveyed station (40°S) where surface Antarctic water occupied the upper 15 m.

- *C. laticeps* was reported by Frost and Fleminger (1968) as an antarctic-subantarctic, circumglobal species occurring at >40°S. During AMT-15, it occurred at 40°S where surface Antarctic water occupied the upper 15 m.

- *C. lividus* was reported by Frost and Fleminger (1968)as a subtropical, circumglobal species. The northern limit in the Pacific Ocean was 50°N (Frost and Fleminger, 1968), while in the Atlantic Ocean was 57.5°N (Williams and Wallace, 1975). The southern limit was 40°S in the Pacific Ocean and 35°S in the Atlantic Ocean according to Frost and Fleminger (1968). These authors highlighted that *C. lividus* was apparently antitropical in

the Pacific Ocean but their data were insufficient to say whether it was also absent from equatorial waters of the other oceans. During AMT-15, *C. lividus* was observed in the North Atlantic (46°-38.9°N) and in the South Atlantic (25.2°-34.5°S) with a clear antitropical distribution also in the Atlantic Ocean.

- *C. mastigophorus* was reported by Frost and Fleminger (1968) as a tropical-subtropical, circumglobal species, with the northern limit at 35°N while Williams and Wallace (1975) reported it up to 47.5°N. The southern limit reported by Frost and Fleminger (1968) was 35°S in South African meridional coast. During AMT-15, this species was sporadically recorded from 48°N to 28.5°S.

- *C. ingens* was reported by Frost and Fleminger (1968) as a warm temperate, southern hemisphere, circumglobal species, ranging from 30°S to 50°S. During AMT-15, it was recorded only twice but always in the South Atlantic (at 28.5°S and 34.5°S).

The distribution patterns of *Clausocalanus* species analysed in relation to environmental parameters allowed to discern the biogeochemical provinces described by Longhurst *et al.* (1995); Longhurst (2006) despite using data from the upper 200 m rather than from the surface. The biogeochemical provinces could not be discerned from distribution of copepod genera (Woodd-Walker, 2001), while species distribution allowed such fine regional subdivision as the author already suggested. Ecological requirements differ among species even when they are congeners inhabiting marine planktonic ecosystems without apparent strong physical barriers.

In synthesis, during the AMT-15, the three smallest *Clausocalanus* species

were the most abundant and showed differences in their distributions (Fig. 4.10): *C. paululus* occurred in temperate oligotrophic waters (NATR and SATL provinces), *C. pergens* in cold eutrophic waters (NADR, CNRY and SSTC provinces), and *C. furcatus* in warm oligotrphic waters (NATR and WTRA provinces). They were followed in abundance by the median-size species of the genus, which also displayed differentiated distributions: *C. jobei* occurred at cold eutrophic onshore sites (NECS and CNRY provinces), *C. arcuicornis* in cold-temperate mesotrophic waters (NADR and NTRA provinces), *C. parapergens* in the upwelling area (CNRY province), and *C. brevipes* and *C. laticeps* were only observed in the Sub-Antartic region (SSTC province). The largest were the less abundant; *C. lividus* occurred mainly in cold mesotrophic waters (NADR province), while *C. mastigophorus* occurred in warm oligotrophic waters (NATR and SATL provinces). *C. ingens* occurred only in the southern hemisphere in temperate oligotrophic waters (SATL-SSTC provinces boundary). Their marked biogeographical distributions and their importance in oceanic waters, *Clausocalanus* species might be considered good indicators of change in copepod communities and of ecosystems modifications due to climate change as Beaugrand *et al.* (2002b) did for copepod associations.

Table 4.2. Studies reporting *Clausocalanus* genus abundance and/or distribution in the Atlantic Ocean published after the revision of Frost and Fleminger (1968).

Author	Latitude	Longitude	Area	Information
Longhurst and Williams, 1979	62°N-39°N	25°-10°W	North Atlantic	Dominance
Beaugrand *et al.*, 2002	60°-40°N	60°-0°W	North Atlantic	Occurrence
Gallienne *et al.*, 2001	60°N-37°N	20°W	North Atlantic	Dominance
Gallienne and Robins, 2001	60°N-37°N	20°W	North Atlantic	Dominance
Head *et al.*, 1999	59° / 37°N	20°W	North Atlantic	Dominance
John *et al.*, 2001	50°N	6°-2°W	English Channel	Occurrence
Morales *et al.*, 1993	49.5°-46°N	19-16°W	North Atlantic	No dominance
Roe, 1984	44°N	13°W	North Atlantic	Dominance
Gil *et al.*, 2002	45°N	4°-3°W	Bay Biscay	Dominance
Valdés *et al.*, 1990	44°-42°N	10°-9°W	Bay Biscay	Dominance
Villate *et al.*, 1997	44°-43°N	2°W	Bay Biscay	Occurrence
Reiss *et al.*, 2002	42°N	62°W	NW Atlantic	Dominance
Head *et al.*, 2002	38°/35°/30°N	20-23°W	Azores	Dominance
Huskin *et al.*, 2001	37°-28°N	20-23°W	Azores	Dominance
Huskin *et al.*, 2004	37°-28°N	20-23°W	Azores	Dominance
Deevey, 1971	32°N	64.5°W	Sargasso Sea	Dominance
Deevey and Brooks, 1971	32°N	64.5°W	Sargasso Sea	Dominance
Calbet and Agustí, 1999	25°N-50°S	70°-30°W	Mid Nord Atlantic-Falkland Is.	Dominance
Barange *et al.*, 1998	40°S	0°E	South Georgia	Dominance

Dominance: genus *Clausocalanus* was reported as a dominant genus in the total copepod assemblage.
Occurrence: genus *Clausocalanus* was reported in the studied area.

Table 4.3. Studies reporting *Clausocalanus* species abundance and/or distribution in the Atlantic Ocean published after the revision of Frost and Fleminger (1968). ARC *C. arcuicornis*, BRE *C. brevipes*, FAR *C. farrani*, FUR *C. furcatus*, JOB *C. jobei*, ING *C. ingens*, LAT *C. laticeps*, LIV *C. lividus*, MAS *C. mastigophorus*, MIN *C. minor*, PARA *C. parapergens*, PAU *C. paululus*, PER *C. pergens*.

Author	Latitude	Longitude	Area	Information
Williams and Wallace, 1975	60°-45°N	60°-0°W	N Atlantic and North Sea	Annual cycle of PER, FUR, JOB, PARA, ARC, LIV and MAS
Piontkovski *et al.*, 2006	60°-15°N	60°-0°W	North Atlantic	Dominance of FUR
Turner, 1981	50°-20°N	80°W	N.America coast	Occurrence of FUR under 30°N
Castel and Courties, 1982	44°N	1°W	Bay in France	Occurrence of ARC
Ribera-Maycas *et al.*, 1999	44°N	2°W	Bay Biscay	Diel vertical distribution of ARC
Álvarez-Marques, 1979	43°N	5°W	Cantabrian coast	Annual cycle of PER, FUR, ARC, JOB, LIV and MAS
Colton, 1962	45°-40°N	68°W	Gulf Maine	Occurrence of ARC
Durbin and Casas, 2006	45°-40°N	64°-71°W	Georges Bank	Dominance of ARC from January to June
Grice and Hart, 1962	45°-30°N	70°W	Gulf Stream	Occurrence of PAU, PER, FUR and ARC
Blanco-Bercial et al., 2006	43°-42°N	9-10°W	UpW Galicia	Occurrence of PER, FUR, JOB, ARC, LIV and MAS
Vieira *et al.*, 2003	40°N	8°W	Esturay in Portugal	Dominance of ARC
Queiroga *et al.*, 2005	40.7°N	9-8°W	UpW Portugal	Occurrence of ARC
Ashjian *et al.*, 1998	38°N	74°W	Mid-Atlantic Bridge	Dominance of PER in winter-spring
Deevey, 1971	32°N	64.5°W	Sargasso Sea	Annual cycle of PAU, FUR and ARC
Schulz, 1986	32°-21°N	55°-70°W	Sargasso Sea	Vertical distribution FUR, PARA, ARC and MAS
Colton, 1975	30°N	70°W	Sargasso Sea	Occurrence of ARC
Schnack-Schiel and Mizdalski, 2002	30°N	28°W	Northeast Atlantic	Occurrence of PAU and FUR
Hernández-León, 1998	28°N	16°W	Canary Island	Annual cycle of ARC
Suárez-Morales and Gasca, 2000	21°-18°N	87°W	Caribbean Sea	Annual cycle of FUR and ARC
Salman and Brenning, 1977	10°-22°N	16°W	North Western African coast	Occurrence of PAU, PER, FUR, JOB, ARC and LIV

Dominance: species reported as a dominant species in the total copepod assemblage.
Occurrence: species reported in the studied area.

Table 4.3 (Continued)

Author	Latitude	Longitude	Area	Information
Lopes *et al.*, 1999	10°S	42°-40°W	SE Brazil	Dominance of FUR in austral winter
Bjornberg, 1980	18°-24°S	-	Brazil current	Distribution of all *Clausocalanus* species except LIV
Valentin and Monteiro-Ribas, 1993	18°-24°S	42°-38°W	Brazil coast	Occurrence of FUR and ARC
Valentin *et al.*, 1987	23°S	42°W	Brazil coast	Dominance of FUR and ARC
Marrari *et al.*, 2004	34°-41°S	52-62°W	Argentina coast	Occurrence of BRE
Berasategui, pers. com.	40°S	62°W	Argentina coast	Occurrence of BRE and LAT
Froneman and Pakhomov, 1998	44-47°S	37°E	Southern Ocean	Dominance of BRE and LAT
Froneman *et al.*, 2002	44-47°S	37°E	Southern Ocean	Occurrence of BRE and LAT
Hunt and Pakhomov, 2003	44-47°S	37°E	Southern Ocean	Occurrence of LAT (offshore) and BRE (shelf)
Hunt *et al.*, 2002	44-47°S	37°E	Southern Ocean	Relationship of LAT with temperature and BRE with Chl *a*
Hunt and Hoise, 2003	48-62S	140°E	Southern Ocean	Occurrence of BRE and LAT
Mayzaud *et al.*, 2002	48°-68°S	62°E	Southern Ocean	Estimation of repiration and ingestion of LAT
Atkinson and Sinclair, 2000	50°-65°S	65°-0°W	Southern Ocean	Vertical distribution of LAT
Mazzocchi and Ianora, 1991	50°S	75°-63°W	Chile fjords	Occurrence of BRE, LAT and ING
Marin, 1987	51-62°S	30-50°W	Southern Ocean	LAT southern limit: Antarctic Convegence Current-Weddell Sea
Atkinson and Sinclair, 2000	50°-65°S	80°-0°W	Southern Ocean	LAT reported as an epipelagic non seasonal migrant species
Chiba *et al.*, 2001	64°S	90-160°E	Southern Ocean	Occurrence of LAT

Dominance: species reported as a dominant species in the total copepod assemblage.
Occurrence: species reported in the studied area.

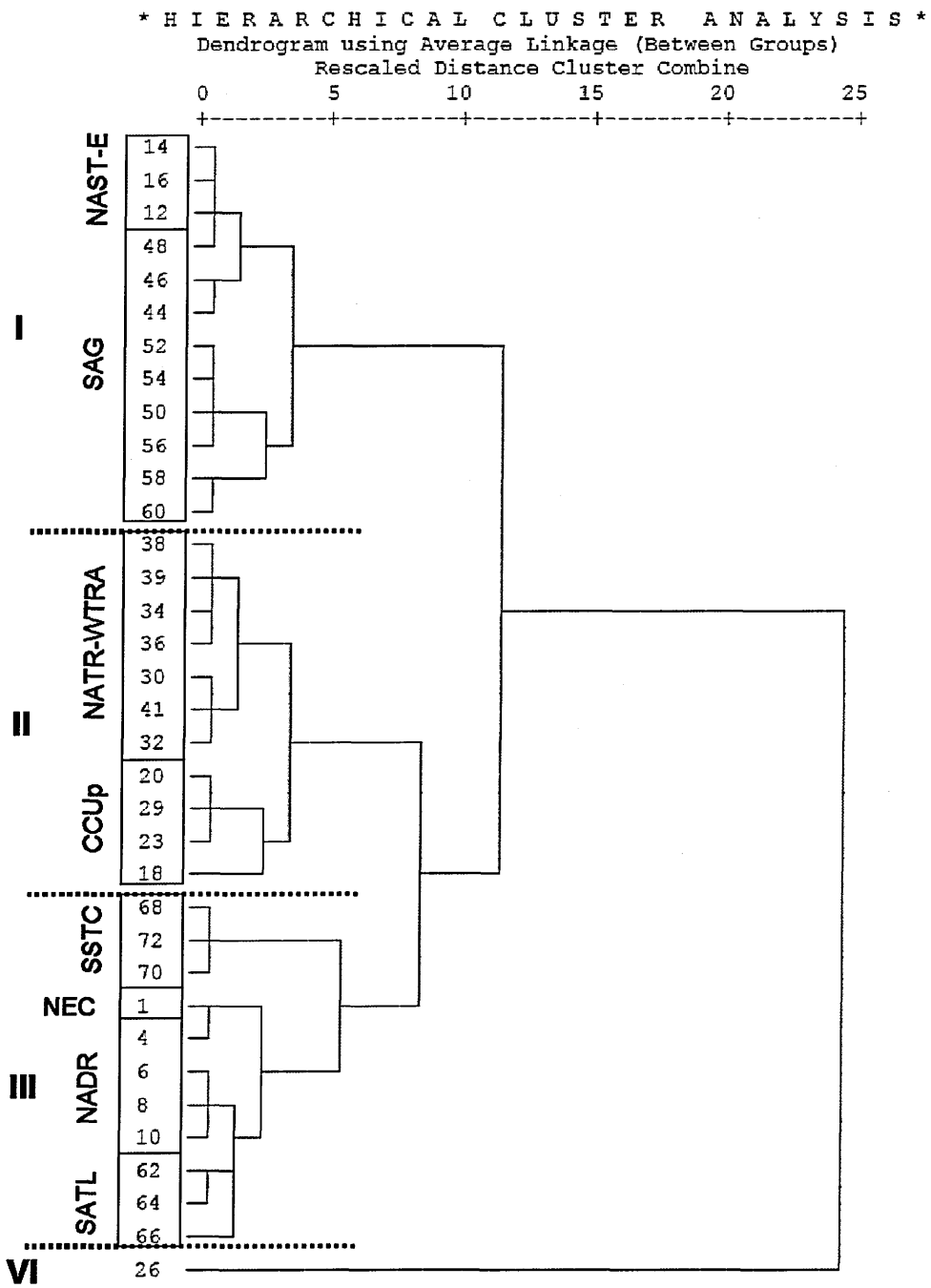

Figure 4.8. Dendrogram from the cluster analysis of the environmental parameters (temperature, salinity and chlorophyll *a* concentration in the mixed layer and DCM depth occurrence) encountered during AMT-15.

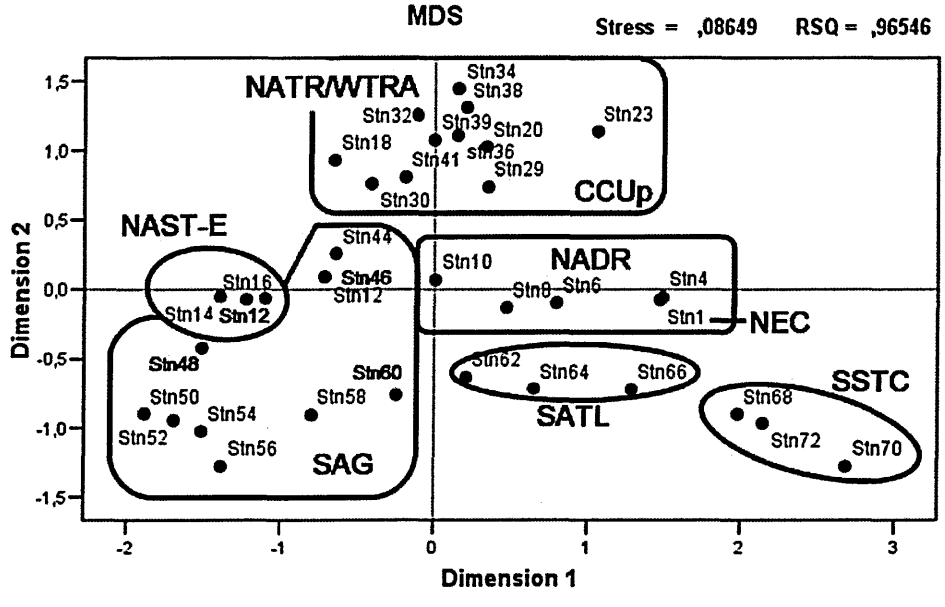

Figure 4.9. Multidimensional scaling analysis of the environmental parameters (temperature, salinity and chlorophyll *a* concentration in the mixed layer and DCM depth occurrence) encountered during AMT-15.

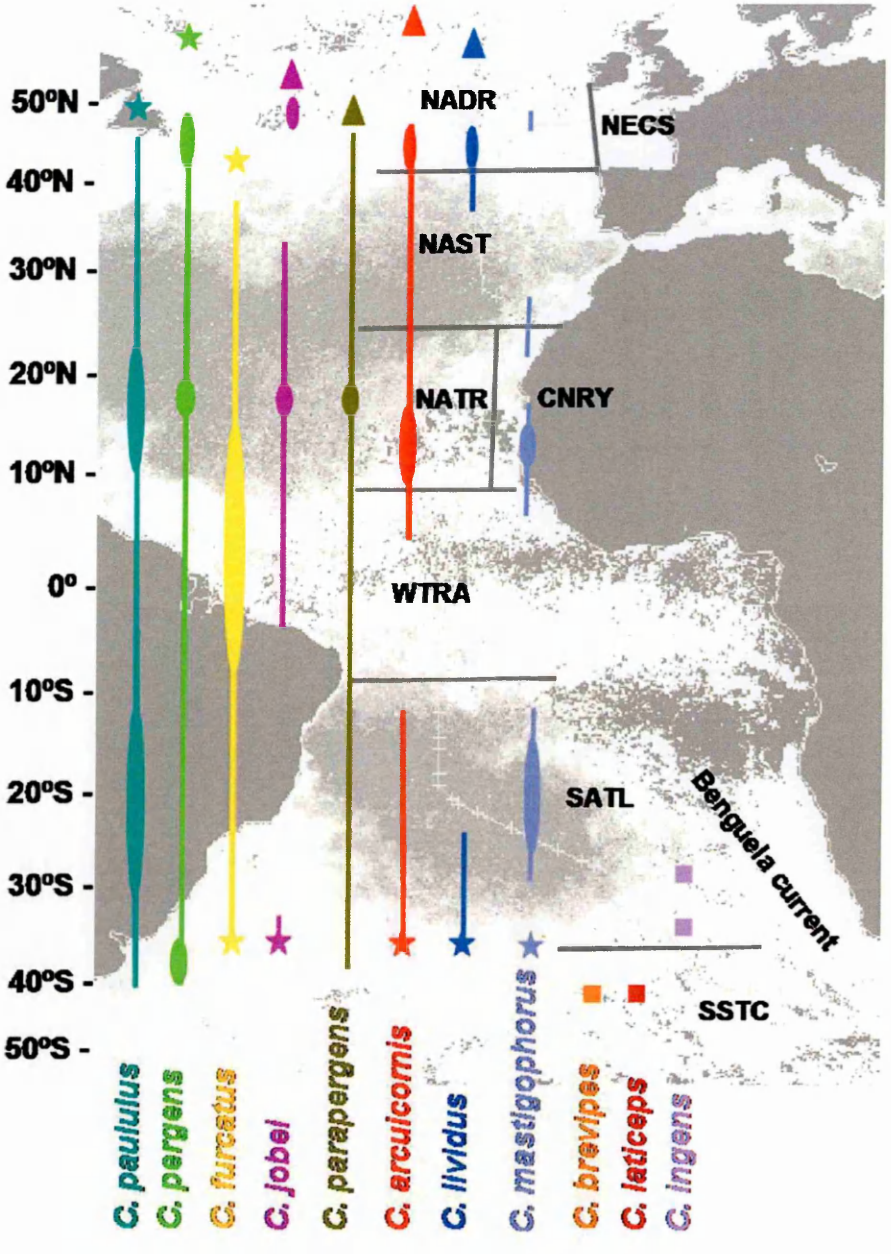

Figure 4.10. *Clausocalanus* latitudinal distribution in the Atlantic Ocean. Each colour correspond to a different species. Lines indicate species latitudinal range of occurrence observed during the AMT-15 cruise (September-October 2004) and widening indicate where their population were concentrated. Squares indicate punctual observations of these species during the cruise. Northernmost or southernmost occurrence recorded in literature are indicated as an star (Frost and Fleminger, 1968) or a triangle ((Williams and Wallace, 1975). Biogeochemical provinces crossed during the cruise according to Longhurst *et al.* (1995) and Longhurst (2006): NECS Northeast Atlantic Continental Shelf; NADR North Atlantic Drift Province; NAST North Atlantic Subtropical Gyral Province; NATR North Atlantic Tropical Gyral Province; CNRY Canary Current Coastal Province; WTRA Western Tropical Atlantic Province; SATL South Atlantic Gyral Province; SSTC South Subtropical Convergence Province.

CHAPTER 5

Niche characterization

The niche characterization of eight *Clausocalanus* species in relation to the major environmental parameters has been attempted on the basis of the whole data set of *Clausocalanus* distribution in different Mediterranean and Atlantic regions that has been presented and discussed in Chapters 2-4.

For this goal, the total 237 copepod samples were analyzed all together with concomitant depth integrated environmental parameters, from which only temperature and fluorescence were considered. Temperature is known to be the major factor affecting species metabolism and development in copepods (Huntley and Lopez, 1992) and fluorescence is the proxy for autotrophic biomass that has been recorded in all surveys of the present study and can be considered representative of potential food available for *Clausocalanus*. For this final synthesis, only the adult female abundances have been taken into account, because they were the population component that has been identified at the species level in all samples.

For each species, data of abundance were first plotted using kriging (a geostatistic analysis) against the integrated values of temperature and fluorescence recorded by CTD in the sites of their occurrence in the different regions explored to obtain a three dimensional representation of the species distribution. With the boxplot method (Tukey, 1977), the distribution (presence/absence) of each *Clausocalanus* species was represented in relation to temperature or fluorescence, separately, as a measure of the range of conditions under which the species can persist (i.e., the niche breadth). For each species, a box and whisker plot is drawn:

the bottom and the top of the box are respectively the 25^{th} and 75^{th} quartiles of the range, whereas the line inside it is the median. The whiskers extending from the two ends of the box show the extent of the rest of the data. Values beyond the end of the whiskers are the outliers (open circles; cases with values between 1.5 and 3 box lengths) and extreme values (asterisks; cases with values more than 3 box lengths). The box length is the interquartile range, also referred to as dispersion (in a statistical sense). Two samples can be considered statistically similar when the median lines overlap each other and the dispersions are comparable. Species abundance were taken into account by using one of the most simple and common approaches for inferring environmental conditions: weighted averaging. This calibration data set was then used to identify the environmental conditions under which different species are most likely to be found (i.e., the species optima). Weighted averaging has been shown to provide accurate inferences of environmental conditions in a wide variety of settings (ter Braak and Barendregt, 1986). Data were statistically analysed by utilizing the SPSS package.

The species environmental ranges of occurrence largely overlapped (Fig. 5.1; 5.2; 5.3; 5.4; Table 5.1) while the species optima (i.e., the environmental conditions under which a species largely occurs) differed considerably (Fig. 5.5).

C. paululus and *C. pergens* showed the largest ranges of occurrence and the same niche breath; both extended their distributions from 10.1 to 22.9°C and from 0.0 to 7.3 f.u. but *C. paululus* had its highest peaks of abundance at higher temperature and lower fluorescence values (15.3 ± 0.08°C, 0.5 ± 0.01 f.u.), while *C. pergens* had its optima in colder and richer waters (13.7 ± 0.18°C, 1.14 ± 0.02 f.u.). *C. paululus* was the most widespread species both in the Mediterranean and in the Atlantic, while *C. pergens* was mainly restricted to cold eutrophic areas. *C. furcatus* had similar temperature range but at higher temperatures than its

similar in size congeners (13.0-25.3°C) while it occurred in narrower fluorescence range (0.0-3.0 f.u.), so it had narrower trophic niche breath. *C. furcatus* had the highest optimum temperature values and low optimum fluorescence values (21.7 ± 0.33°C, 0.37 ± 0.01 f.u.).

Among the medium-sized *Clausocalanus* species, *C. parapergens* had the narrowest temperature and fluorescence ranges (13.0-22.9°C, 0.0-1.8 f.u.). *C. jobei* had slightly wider occurrence ranges (13.0-23.9°C, 0.0-3.0 f.u.) than *C. parapergens*. *C. arcuicornis* had the largest temperature and fluorescence ranges (13.0-25.3°C, 0.0-7.3 f.u.) and so it had larger niche breath. Optima conditions slightly differed among species: *C. jobei* optima values were 16.9 ± 0.21°C and 0.34 ± 0.01 f.u., *C. parapergens* optima values were 15.9 ± 0.15°C and 0.15 ± 0.01 f.u., while *C. arcuicornis* optima values were 15.6 ± 0.15°C and 0.46 ± 0.01 f.u.

The large species *C. lividus* and *C. mastigophorus* had similar temperature ranges of occurrence (12.8-22.5°C and 13.1-22.9°C, respectively) while *C. lividus* fluorescence range was larger than that of *C. mastigophorus* (0.0-7.3 f.u. and 0.0-1.7 f.u., respectively). *C. lividus* temperature optimum was lower than *C. mastigophorus* while fluorescence optimum was higher (14.7 ± 0.15°C and 17.2 ± 0.15°C, and 0.93 ± 0.02 f.u. and 0.24 ± 0.00 f.u., respectively).

Results from the ANOVA tests were summed up in Table 5.2 and showed that the niche breath of *Clausocalanus* species in relation to temperature were significantly different between *C. furcatus* and all the other species except *C. mastigophorus*; and between *C. mastigophorus* and *C. pergens* and *C. paululus*. For fluorescence ranges, no significant differences were found between species.

The female distribution might represent only a partial sector of species distribution, which can be slightly different for males and juveniles. However, in *Clausocalanus* species, males are much less abundant than females, and copepo-

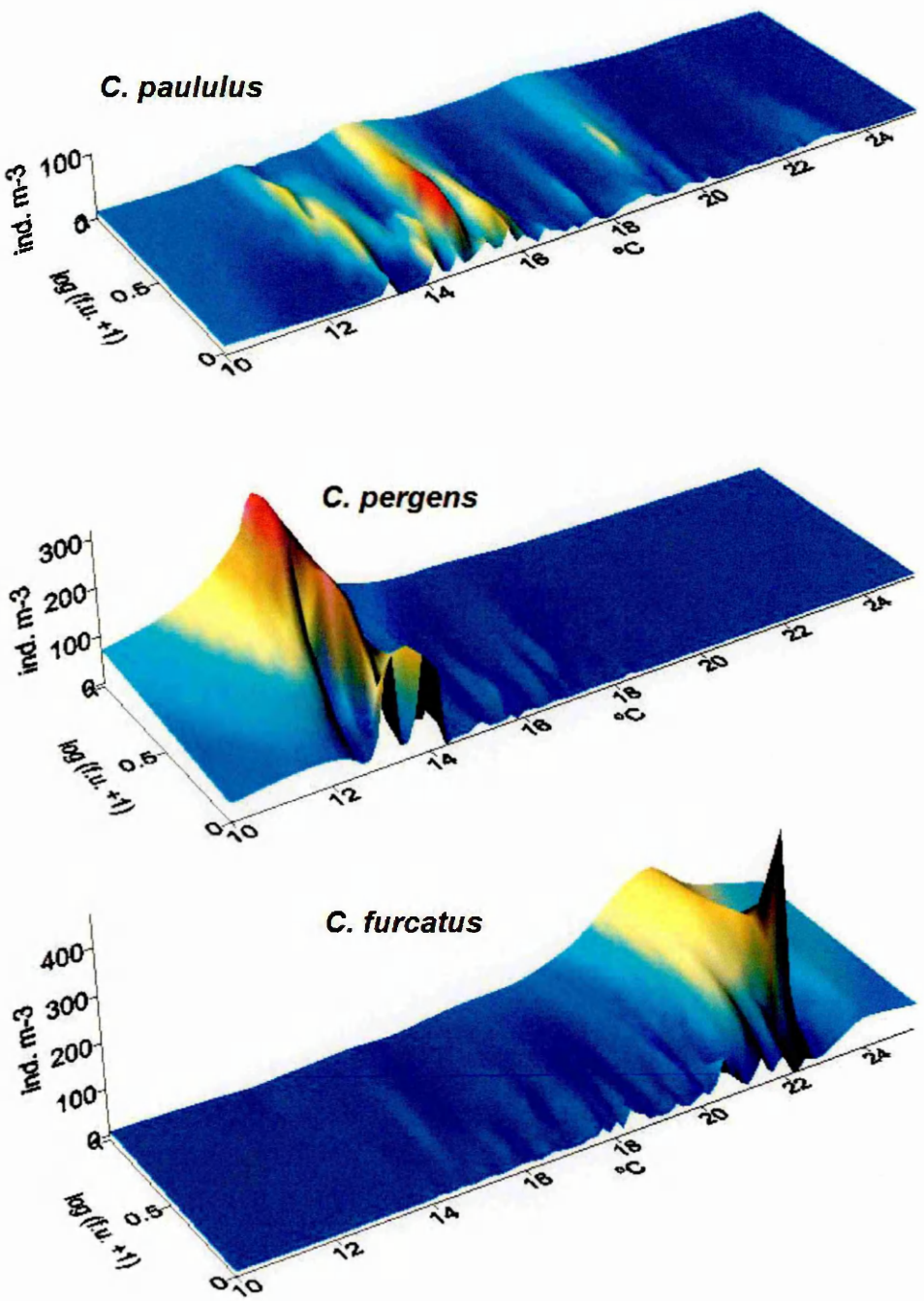

Figure 5.1. 3-D representation of small-sized *Clausocalanus* species abundances in relation to temperature (°C) and fluorescence ($\log_{10}$ (f.u.+1)). Abundances and integrated environmental data collected in: the Gulf of Naples (Stn MC 0-50 m and Stn L20 at discrete layers), the Ionian Sea (0-100 m), the North Western Mediterranean (0-50, 50-100 and 100-200 m) and the Atlantic Ocean (0-200 m). Colour gradient is only indicative, changing from blue when the species abundance was minimum to red when its abundance was maximum.

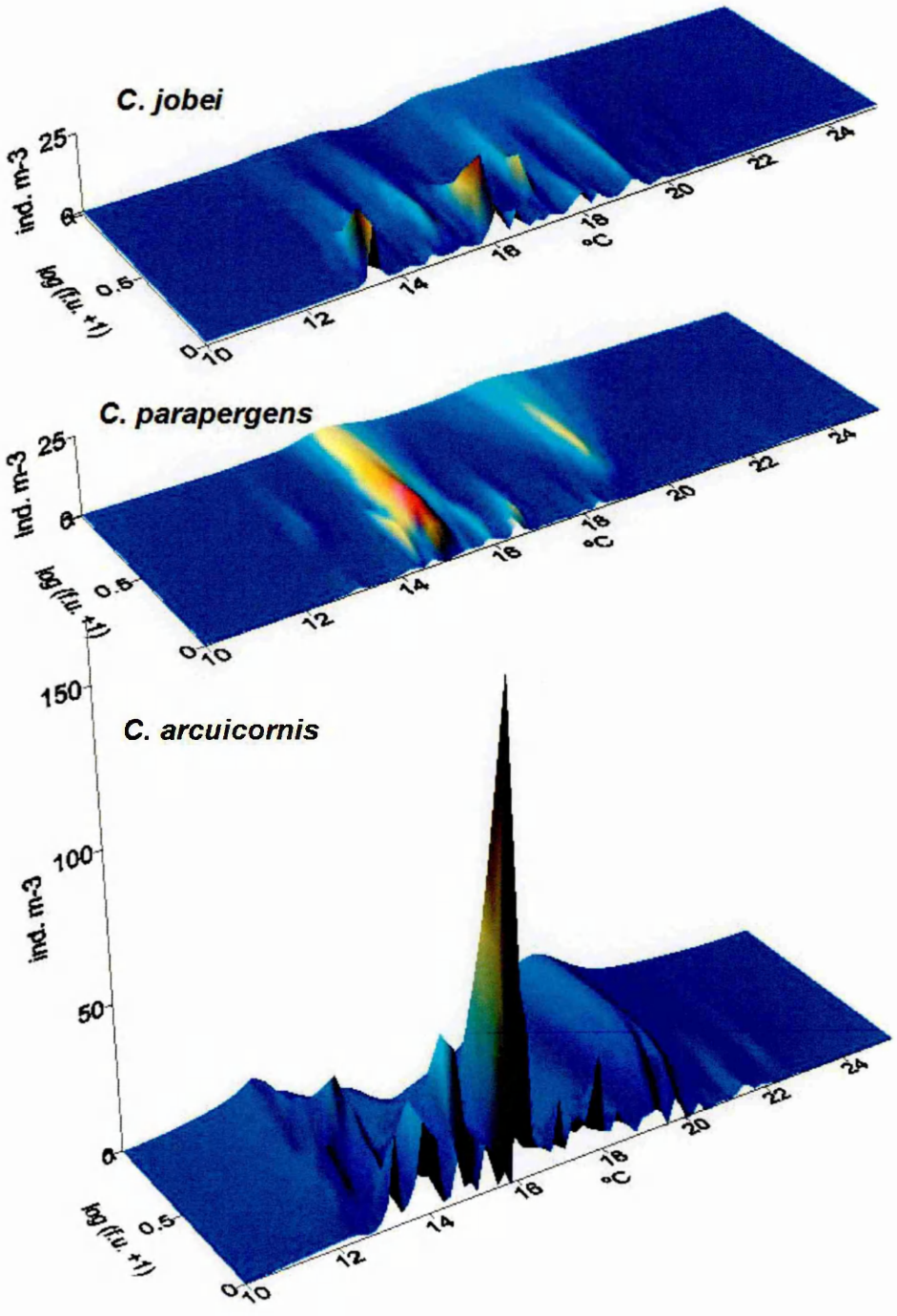

Figure 5.2. 3-D representation of medium-sized *Clausocalanus* species abundances in relation to temperature (°C) and fluorescence ($\log_{10}$ (f.u.+1)). Abundances and integrated environmental data collected in: the Gulf of Naples (Stn MC 0-50 m and Stn L20 at discrete layers), the Ionian Sea (0-100 m), the North Western Mediterranean (0-50, 50-100 and 100-200 m) and the Atlantic Ocean (0-200 m). Colour gradient is only indicative, changing from blue when the species abundance was minimum to red when its abundance was maximum.

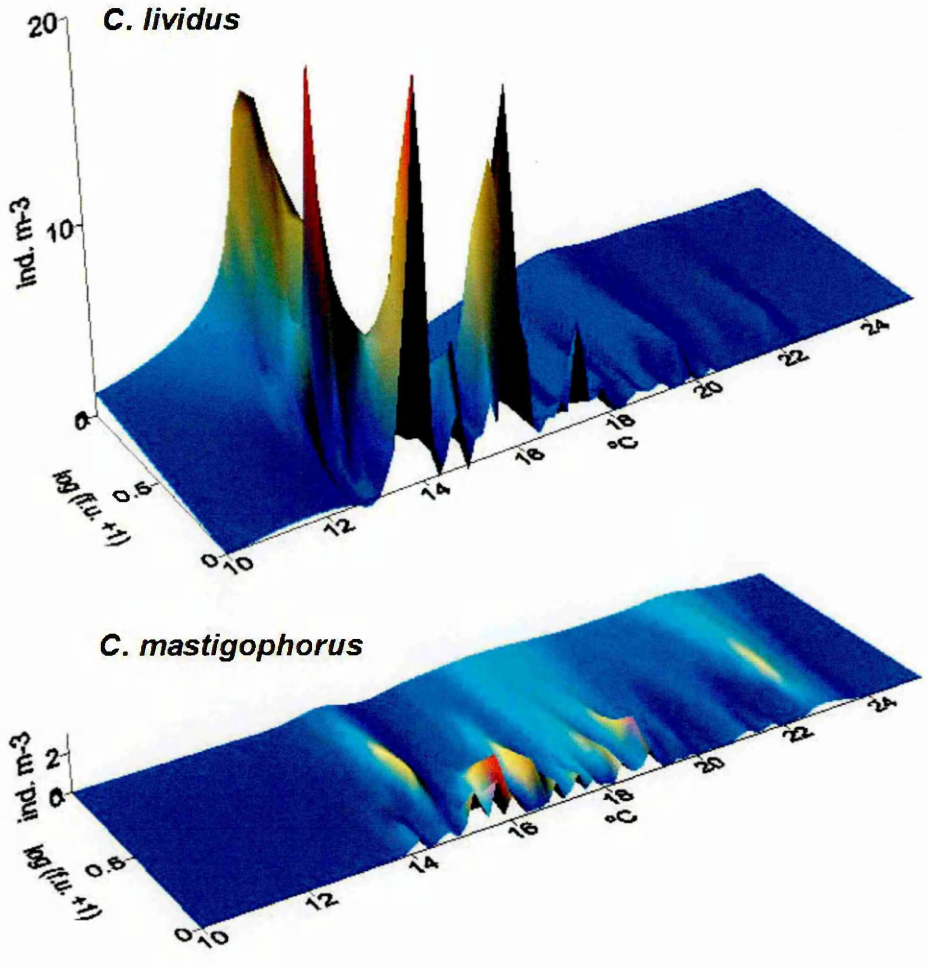

Figure 5.3. 3-D representation of large-sized *Clausocalanus* species abundances in relation to temperature (°C) and fluorescence ($\log_{10}$ (f.u.+1)). Abundances and integrated environmental data collected in: the Gulf of Naples (Stn MC 0-50 m and Stn L20 at discrete layers), the Ionian Sea (0-100 m), the North Western Mediterranean (0-50, 50-100 and 100-200 m) and the Atlantic Ocean (0-200 m). Colour gradient is only indicative, changing from blue when the species abundance was minimum to red when its abundance was maximum.

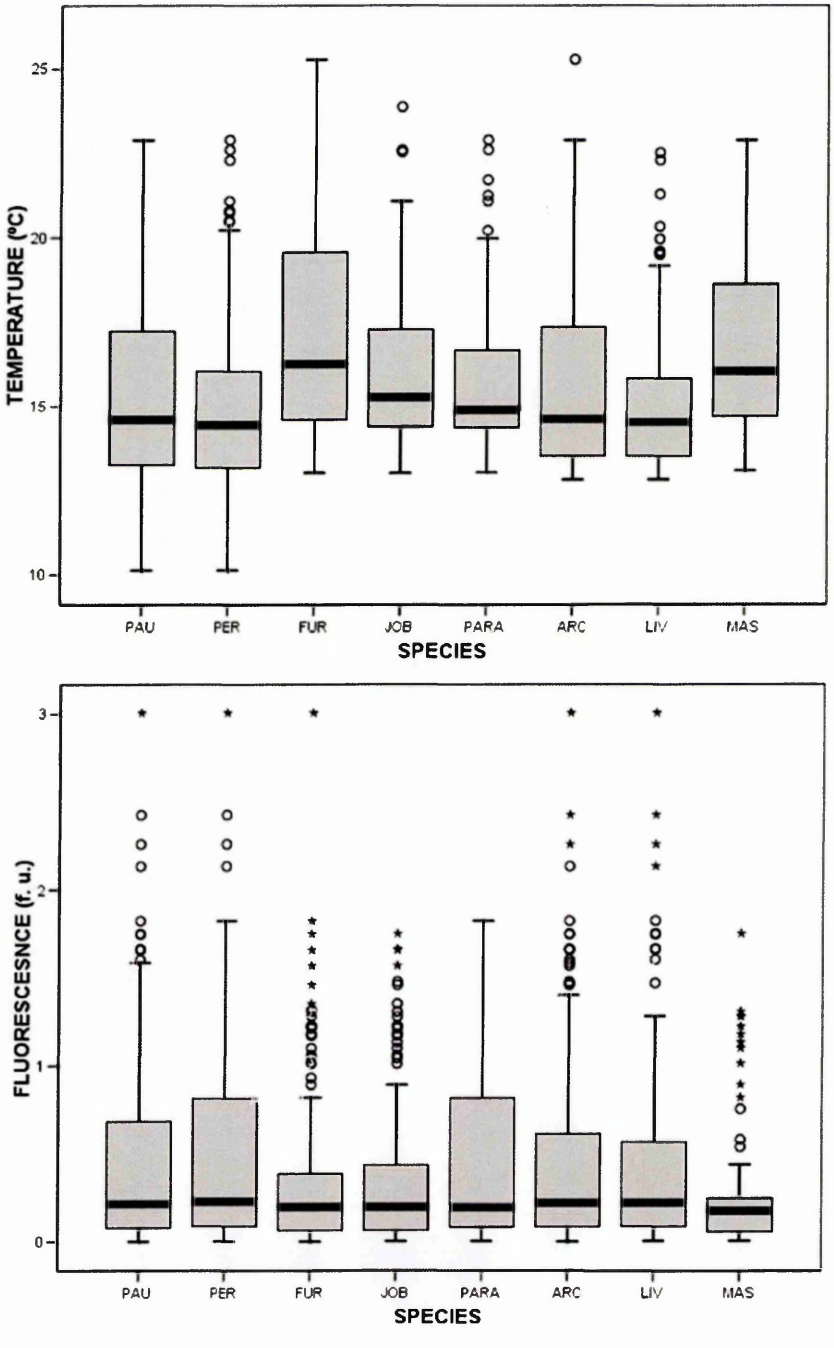

Figure 5.4. Environmental ranges (temperature and fluorescence) of *Clausocalanus* species occurrence (niche breadth) in the Mediterranean Sea and in the Atlantic Ocean, represented in a box and whisker plot. The bottom and the top of the box are respectively the 25^{th} and 75^{th} quartiles of the range, whereas the line inside it is the median. The whiskers extending from the two ends of the box show the extent of the rest of the data. Values beyond the end of the whiskers are the outliers (open circles; cases with values between 1.5 and 3 box lengths) and extreme values (asterisks; cases with values more than 3 box lengths).

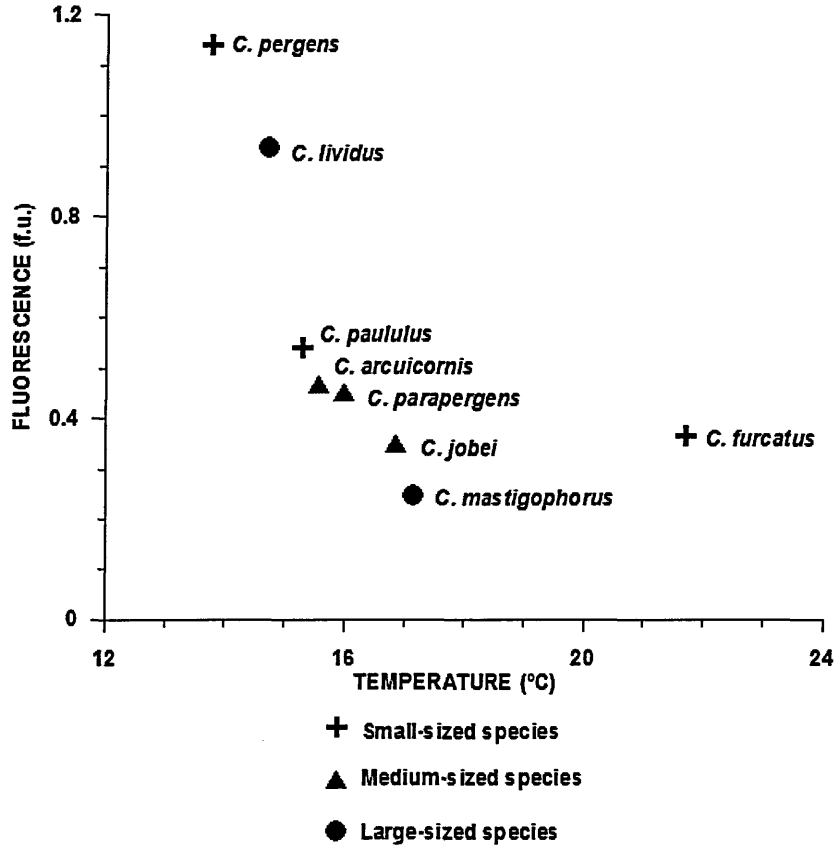

Figure 5.5. Environmental conditions (temperature and fluorescence) under which different *Clausocalanus* species are most likely to be found (i.e., the species optima). Such conditions were inferred from weighted averaging abundance data collected in: the Gulf of Naples (Stn MC 0-50 m and Stn L20 at discrete layers), the Ionian Sea (0-100 m), the North Western Mediterranean (0-50, 50-100 and 100-200 m) and the Atlantic Ocean (0-200 m).

dids (which in general are likely more sensitive to environmental excursions in comparison to adults and therefore are not expected to occur at the extremes ranges of population occurrence) were identified to genus level. It seems therefore reasonable to consider that the female distribution is a reliable representation of the species distribution.

In conclusion, the detailed analysis of the temporal and vertical occurrence of *Clausocalanus* species in relation to environmental parameters allowed me to draw

a coherent picture of their distribution and make hypotheses regarding differentiation of their niches. The small species *C. paululus* and *C. pergens*, despite having the same ranges of occurrence (niche breath), differed in the numerical development of their populations and in their optimal environmental conditions. The optimal conditions corresponded to colder (13°C) eutrophic waters for *C. pergens*, and to warmer (15°C) oligotrophic waters for *C. paululus*. *C. furcatus* differentiated from the other species and showed a narrow and quite isolated ecological niche in warm (22°C) oligotrophic conditions. The medium sized species *C. arcuicornis*, *C. parapergens*, and *C. jobei* presented similar seasonal cycles and latitudinal distribution, so they overlapped on their environmental ranges (niche breath). It seems that these species had similar optima conditions but they occurred in different depth layer (i.e., *C. parapergens* peak of abundance occurred deeper in the water column at Stn L20 in the Gulf of Naples) and in different regions (i.e., *C. jobei* occurred mainly in onshore sites while the other two species at offshore sites). The large *C. mastigophorus* and *C. lividus* overlapped in seasonal and vertical distribution in the Mediterranean Sea while in the Atlantic Ocean they occurred over different latitudinal ranges. Optimal conditions were cold eutrophic waters for *C. lividus* and warm oligotrophic waters for *C. mastigophorus*.

Table 5.1. Mean ($\pm$SD) and difference between maximum and minimum values (M-m) of the environmental parameters (temperature, fluorescence and salinity) where *Clausocalanus* species occurred in the Mediterranean and Atlantic waters.

	Temperature		Fluorescence		Salinity	
Species	Mean (SD)	(M-m)	Mean (SD)	(M-m)	Mean (SD)	(M-m)
C. paululus	15.67 (2.70)	12.8	0.55 (0.95)	7.3	37.87 (0.86)	4.4
C. pergens	15.17 (2.33)	12.8	0.60 (0.99)	7.3	37.89 (0.86)	4.4
C. furcatus	17.16 (2.82)	12.3	0.37 (0.49)	3.0	37.70 (0.88)	3.7
C. jobei	16.07 (2.34)	10.9	0.38 (0.47)	1.7	37.85 (0.79)	3.5
C. parapergens	15.68 (2.14)	9.9	0.50 (0.81)	7.3	37.85 (0.90)	3.9
C. arcuicornis	15.75 (2.75)	12.5	0.55 (0.95)	7.3	37.94 (0.73)	3.7
C. lividus	15.21 (2.16)	9.7	0.63 (1.20)	7.3	38.07 (0.57)	3.2
C. mastigophorus	16.88 (2.49)	9.8	0.28 (0.38)	1.7	37.76 (0.85)	3.5

Table 5.2. Scheffer post hoc results indicating significant differences ($p<0.01$) among *Clausocalanus* species niche breaths on temperature ranges. Species did not differed in fluorescence ranges of occurrence. pau *C. paululus*, per *C. pergens*, fur *C. furcatus*, job *C. jobei*, par *C. parapergens*, arc *C. arcuicornis*, liv *C. lividus*, mas *C. mastigophorus*.

	pau	per	fur	job	par	arc	liv	mas
pau		-	*	-	-	-	-	*
per	-		*	-	-	-	-	*
fur	-	-		-	*	*	*	
job	-	-	-		-	-	-	-
par	-	-	-	-			-	-
arc	-	-	-	-	-		-	-
liv	-	-	-	-	-	-		*
mas	-	-	-	-	-	-	-	

CHAPTER 6

Clausocalanus reproductive parameters and secondary production

6.1. Materials and methods

Mesozooplankton samples for collection of experimental copepods were taken using 200 μm mesh nets equipped with non-filtering cod-ends (5-10 L) to prevent animal damage. Vertical tows were performed during the day (8-12 am) from 50 m depth (Ionian Sea, Gulf of Naples, NW Mediterranean) or at night (2 am) from the layer of deep chlorophyll maximum (Atlantic Ocean) up to the surface. Once onboard, live samples were gently poured into 18 L cooler-boxes previously filled with sea surface water or water from the deep chlorophyll maximum.

Immediately after zooplankton sampling, natural particle assemblages were collected from the sea surface (Gulf of Naples) or from the deep chlorophyll maximum (Ionian Sea, NW Mediterranean and Atlantic Ocean) with Niskin bottles that were drained with long silicon tubes whose end aperture were always resting on the bottom of the collector bin in order to avoid water gurgling and cell dam-

age. Bins were screened from direct light and kept at temperature close to that at the sampling layer and used as incubation water during the experiments within 1-2 hours after sampling.

Mesozooplankton samples were examined within 1 hour after collection. Cooler-boxes were first cleaned from dead settled material. Then, subsamples were poured using 2-5 L glass beakers and examined by eye. *Clausocalanus* species are easily distinguishable from other copepods by their particular swimming behavior (Mazzocchi and Paffenhöfer, 1999). They were caught using a long large-mouth glass pipette, transferred to a small petri dish and checked under the stereoscope for species identification and conditions. Then, using a wide mouth Pasteur pipette, only healthy females were introduced into the incubation flasks. The particular reproductive features of *Clausocalanus* species required two different incubation methodologies in order to study the reproductive parameters of sac spawners and free-spawners efficiently. The more abundant species were studied at each site, except for *C. paululus* which was very difficult to recognize and catch.

Clausocalanus females that carried egg-sac/mass were sorted and put individually in a small Petri dish in a drop of seawater where the surface tension forced the copepod to slowdown or stop its frantic motion. In order to measure the clutch size, the sac/mass was carefully detached using dissecting needles and the eggs were counted. After that, females were incubated individually in 300 ml polystyrene cell culture flasks and monitored for further clutch depositions. Untreated sterile polystyrene cell culture flasks are made of high-quality polystyrene for optical clarity and distortion-free examination and are designed to be easily checked under the stereoscope avoiding stressing the animal. The incubation flasks were previously filled with non-filtered sea water containing natural particle assemblages (but not other copepods that were pick up from the experimental

flasks with a pipette). Females with spermatophore(s) were incubated individually immediately after sorting in 300 ml flasks. Females that were not bearing a sac or spermatophores, were incubated in 2 L glass jars or bottles at 10-20 ind L^{-1} concentration.

Clausocalanus free-spawning females were individually incubated in 300 ml polystyrene culture flasks filled with pre-screened sea water (40 μm sieve). Despite reducing the size range of pray available to copepods, water screening ensured the removal of other copepod eggs.

Incubation water was poured into the experimental containers using a plastic jug performing slow and gentle movements to avoid cell damage by water gurgling. *Clausocalanus* specimens usually get trapped in the surface film where they move frantically, a stressful situation with consequent animal death. To avoid this, as well as to prevent air bubbles forming in the experimental containers, they were completely filled and sealed with plastic film under the screw cap.

All experimental containers were maintained at sea surface temperature encountered during zooplankton collection. Different methods were used for this purpose: placing bottles and flasks in temperature-controlled coolers (Ionian Sea) or rooms (Gulf of Naples and tropical region in the Atlantic Ocean), immersing bottles and flasks in thermostated incubators (NW Mediterranean) or immersing bottles and flasks in fresh water directly pumped from sea surface (Atlantic Ocean, except in the tropical region where sea surface temperature exceed 30°C and incubated copepods died).

In order to avoid settling, experimental containers were gently and regularly turned upside down (Ionian Sea, NW Mediterranean and tropical Atlantic region) or placed on a rotating plankton wheel (set at 0.2 rpm, Gulf of Naples) or placed on a seawater pumped wheel (Atlantic Ocean).

All the experimental containers were incubated under dim light simulating the natural diel cycle.

In order to observe multiple clutch events, incubations lasted as long as possible. Every day, copepods were transferred into new incubation flasks filled with new particle assemblage collected with Niskin bottles from the DCM within the previous hour for providing fresh food (only in onboard experiments). In those experiments carried out in the Gulf of Naples, in 2002-2003 females were incubated only for 24 hours, in April 2006 copepods were reared as long as possible changing the natural particle assemblage of the first day was subsequently replaced by filtered seawater enriched with *Prorocentrum minimum* (16 x 16 μm, 1340 μm^{-3}; cultured and kindly provided by F. Esposito, Ecophysiology Laboratory at SZN). Estimated carbon content of this dinoflagelate is 177.1 pgC $cell^{-1}$ (Strathmann, 1967). *P. minimum* was offered at 173 cells ml^{-1} concentration (30.65 μg C L^{-1}). Offered carbon concentration was intermediate between the low values recorded at sea in the Gulf of Naples in April (Ribera d'Alcalà *et al.*, 2004) and that reported in previous laboratory incubation of *Clausocalanus* (Mazzocchi and Paffenhöfer, 1998; Paffenhöfer *et al.*, 2006). Mazzocchi and Paffenhöfer (1998) observed that lower food concentrations were related to higher egg production rate in *C. furcatus* (in that study, 55 μg C L^{-1} of *Prorocentrum micans* were offered). On the other hand, *C. furcatus* metabolic needs were met even in tropical oligotrophic waters (6.29 ± 0.75 μg C L^{-1} in natural particle assemblage Paffenhöfer *et al.*, 2006).

New food supply was offered every day and water was changed every 2-3 days in accordance with new water supply. When cultured food is offered, the first clutch can be considered as the result of *in situ* individual feeding history. So, in those experiments performed in the Gulf of Naples in April 2006, the first 48 h has been be considered as the result of the *in situ* individual feeding history and

therefore comparable to those experiments performed under *in situ* conditions (onboard experiments). All experiments were held in a very similar way.

Reproductive parameters

Clutch size (CS) — Clutch size is the number of eggs released in each single clutch event. It is expressed as number of eggs per clutch per female (eggs clutch^{-1} female^{-1}).

Experimental containers were checked by eye or under the stereoscope every 6-12 hours looking for the presence of egg-sac/mass or free eggs. Those females carrying egg sac/mass were gently picked up with a large-bore Pasteur pipette and put in a small Petri dish to detach and count the eggs. The female was then transferred to new incubation water. In the case of free-spawning females, when eggs were observed, the female was picked up and transferred to a little petri dish with filtered sea water to check under the stereoscope the presence of free eggs in the picked water. Immediately after, the female was transferred to a new container filled with fresh incubation medium. Eggs picked up with the female were returned to the original flask with the rest of the clutch. The flask with eggs was then filled with filtered sea water, sealed and placed horizontally for at least 30 min waiting for eggs settling. After that, the whole flask was examined and the eggs counted.

Interclutch period (IP) — This is the lapsed time between two successive clutch laying. It is expressed in hours.

Females were incubated as long as possible to measure their interclutch period, controlling every 6-12 h for sac/eggs deposition and changing the incubation water every day.

Egg production rate (EPR) — This is defined as the number of eggs

produced per female per day (eggs female^{-1} day^{-1}). The whole incubation period was taken into account (except for those experiments conducted in spring 2006 in the Gulf of Naples when only the first 48 h were considered for such calculations).

Hatching success and embryo viability — Hatching success is the percentage of eggs that hatch to nauplii from the total eggs in a clutch (%). Embryo viability is the percentage of eggs showing healthy embryos out of the total eggs in a clutch (%). Embryo viability is assessed using molecular probes before egg hatching.

Hatching success is easily calculated in broadcast spawner copepods by allowing eggs to hatch undisturbed. Unhatched eggs and nauplii were counted under the stereoscope after 24 h of being laid and compared to the previous record of eggs. Only in those experiments run in the NW Mediterranean, eggs and nauplii were fixed 24 h after being laid for further counts in the laboratory.

In sac spawners *Clausocalanus* species, the number of eggs cannot be accurately counted without detaching the sac or mass. Mazzocchi and Paffenhöfer (1998) observed that detachment from females may prevent embryos from hatching in *C. furcatus*. Therefore, egg production rates and recruitment could be underestimated in sac spawners *Clausocalanus* either by difficutlies in counting the eggs or for the impedance of hatching in detached sacs or masses. To measure both clutch size and hatching success in such species, a method was set up in collaboration with Dr. I. Buttino (Ecophysiology Laboratory, SZN) using fluorescent probes already successfully applied to the free-spawning copepod *Calanus helgolandicus* (Buttino *et al.*, 2004a). Two probes were tested, FDA and SYTOX green.

Fluorescein diacetate (FDA) (Sigma-Aldrich) is a non-polar, non-fluorescent fluorescein analogue which can pass through the cell membrane where upon in-

tracellular esterases cleave off the diacetate group producing highly fluorescent fluorescein (http://www.sigmaaldrich.com). The fluorescein will accumulate in cells which possess intact membranes so the green fluorescence can be used as a marker of cell viability. Cells which do not possess an intact cell membrane or an active metabolism may not accumulate the fluorescent product and therefore do not exhibit green fluorescence.

SYTOX green (Sigma-Aldrich) is impermeant to live cells and apoptotic cells, but stains necrotic cells with intense green fluorescence by binding to cellular nucleic acids. After staining a cell population with SYTOX green dye, dead cells show green fluorescence and live cells show no fluorescence.

FDA and SYTOX green dyes cannot be used together due to antagonistic results and similar fluorescence (Fig. 6.1).

To set up the method in sac spawners *Clausocalanus*, wild ovigerous *C. furcatus* females were separated in three groups: two groups were placed in glass bottom WillCo-dishes® and stained one with FDA (4.5 μM) and the other with SYTOX green (20 μM). Females of the third group were incubated individually in 300 ml flask and left undisturbed until hatching of nauplii (Control). After 20 minutes, females in treated groups were carefully rinsed. Eggs were carefully detached from females with dissecting needles and observed in epifluorescent, confocal and transmitted-light modes, under the inverted confocal laser scanning microscope (CLSM-Zeiss 410) using 488 nm λ excitation laser and with water immersion objective 25x. In the control group, the whole flask content was fixed in 4% formaldehyde after 24 h, and nauplii and unhatched eggs were counted under the dissecting microscope to estimate the total egg production and embryo viability assuming no cannibalism of eggs.

The new method set up was presented at the 37^{th} CIESM Congress (Buttino

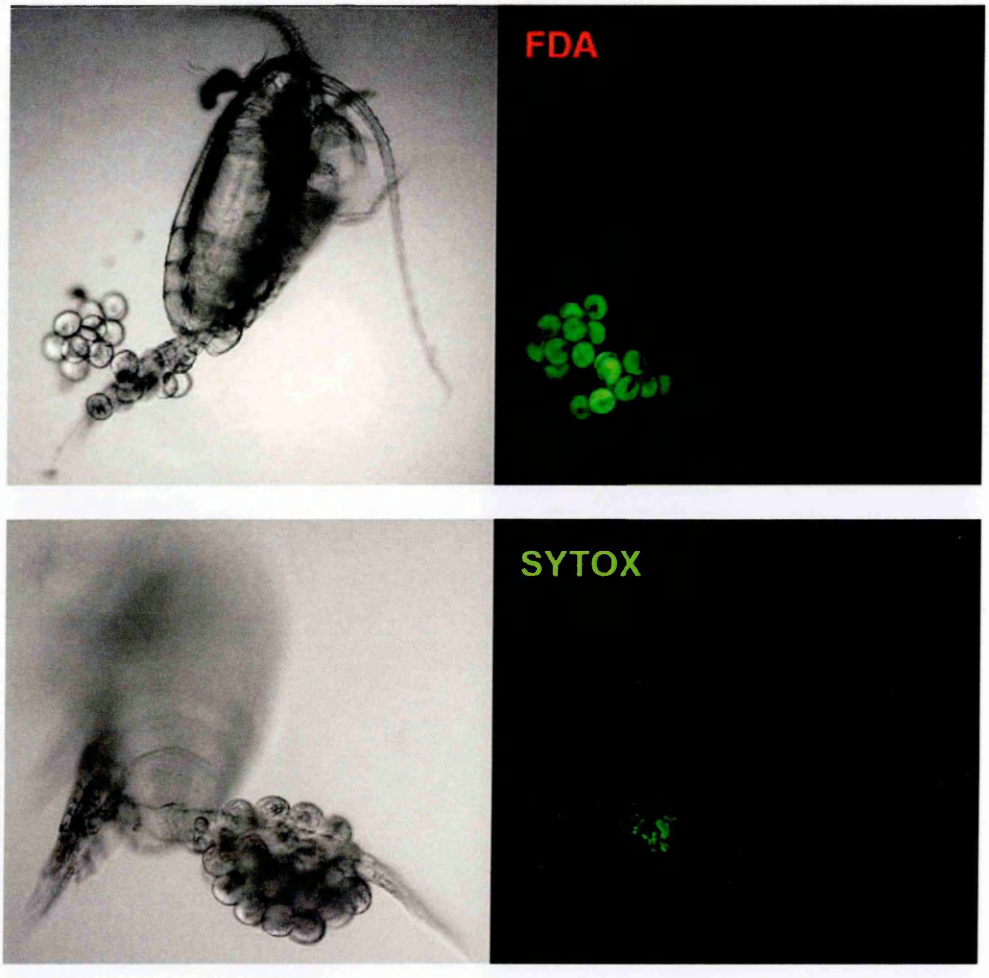

Figure 6.1. *C. furcatus* clutch colored with FDA (upper) and SYTOX green (bottom). Pictures at transmitted light (left) and under green filter (right). All eggs were viable.

et al., 2004b). Data on embryo viability in *Clausocalanus* species were collected during experiments conducted in the Gulf of Naples (2003-2004 and 2006) and in the Atlantic Ocean (2004).

In April 2004, the effect of detaching the sac from females was tested in *C. arcuicornis* using SYTOX green. This experiment was performed overnight, when copepod females laid their eggs (Mauchline, 1998). Females were incubated at 20°C in 2130 ml glass jars, and controlled every 30 min. Ovigerous females

were picked up directly from the jars with a large-mouth glass pipette and divided in three groups: 1) 8 females (486 embryos, Control) were incubated individually and remained undisturbed for 36 h, until embryos hatched naturally; 2) 5 females (300 embryos, T_0) had their egg sacs detached in the first hour after laying; 3) 3 females (169 embryos, T_7) were individually incubated and their egg sacs were detached after 7-8 h from deposition. Detached eggs from groups 2 and 3 were dyed after 60 min of their detachment to ensure enough time to manifest eventual negative effects on the embryos.

In 2006, embryo viability of a free spawner *Clausocalanus* species (*C. lividus*) was assessed using Sytox green. After counting the eggs in the flask (after female removal), the flask contents was poured in 200 ml crystallizers. After 30 min, the eggs (settled on the crystallizers' bottom) were picked up with a Pasteur pipette and transferred in WillCo-dishes® to proceed with their coloration as reported above for sac spawners *Clausocalanus* species.

Egg fertilization — Even non-fertilized eggs can fluoresce with FDA because they are live cells. In order to avoid "false positives" (false viable embryos) another vital probe (Hoechst 33342) was used in parallel with those used to assess embryo viability (in this case SYTOX green)(Fig. 6.2).

Hoechst 33342 is a UV-excitable nucleic acid stain readily taken up by all cells. In contrast, SYTOX green only enters cells with compromised plasma membranes. The staining pattern resulting from the simultaneous use of these two nucleic acid stains made it possible to distinguish healthy fertilized or unfertilized eggs and dead embryo cells by UV and fluorescence microscopy, respectively. Dyed eggs were incubated for 20 min in dark conditions and then rinsed with GF/F filtered seawater. Samples were observed under the Zeiss Axioskop 2 plus microscope equipped with UV laser FluoArc-BP (364 nm λ) and Fitc and UV filter. Non-

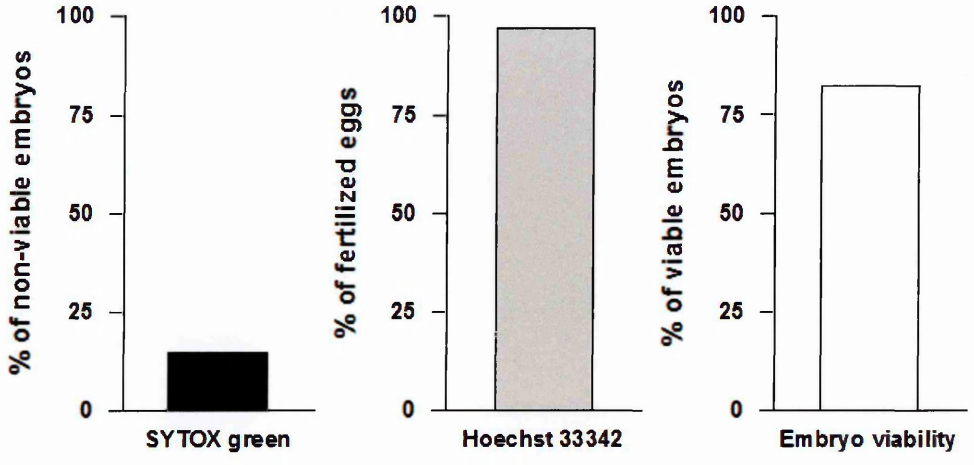

Figure 6.2. A *C. pergens* clutch stained simultaneously with the nucleic acid stains SYTOX green (that indicates embryo non viability when fluorescent) and Hoechst 33342 (that colours all the nuclei present in the egg) revealed five non viable embryos (five fluorescent embryos) and only one unfertilized egg (egg with only one fluorescent nucleus) that was not stained with SYTOX green, confirming the possible occurrence of false viable embryos and so reduced embryo viability.

viable eggs fluoresced green when observed under Fitc filter and all nuclei of the embryo appeared blue under the UV filter. Those eggs showing only one nucleus indicated that they were not fertilized. Egg fertilization was measured in successive clutches in isolated females in order to assess whether re-mating was necessary or not in these species.

Weight-specific fecundity estimates — Egg production rates were converted into carbon content to obtain the weight-specific fecundity rates for a better interspecific comparison.

Female body carbon contents were estimated from prosome lengths measured during the experiments with the length-body carbon regression developed for *Paracalanus* spp. by Uye (1991) and also applied to *Clausocalanus* by Halvorsen *et al.* (2001):

$$log_{10}C(\mu g) = 3.128 * log_{10}PL(\mu m) - 8.451$$

Egg carbon contents were estimated by calculating egg volume from the measured egg diameters by assuming 0.14 pgC μm^{-3} (Kiørboe *et al.*, 1985; Huntley and Lopez, 1992).

Weight-specific fecundity (i.e. weight-specific EPR; g, d^{-1}) was estimated as follows:

$$g = W_e * CS/W_f * 24/t$$

which is the same as,

$$g = W_e/W_f * EPR$$

where W_e represents the weight of a single egg, CS is the clutch size so $W_e * CS$ is the clutch weight, W_f is the female weight and t is the time of incubation in hours. $CS * 24/t$ in the first equation is the EPR in the second (Peterson *et al.*, 2002; Hirst and Bunker, 2003).

In broadcast spawners egg production depend only on ovarian development time to lay a successive clutch while for egg-carrying copepods embryonic development and hatching must occur before a new clutch can be laid. Nevertheless, long incubation periods were required for both spawning modes in order to obtain reliable estimates of egg production since *Clausocalanus* broadcast species do not release eggs continuously or as clutches on a daily basis like most other broadcast copepods (Mauchline, 1998) but showed long interclutch periods. In *Clausocalanus* egg-carrying species, eggs were too packed to enumerate reliably for live females and despite gentile plankton nets employed, eggs were dislodged during the collection process and scattered loose into the sample. Loose eggs can no longer be attributed to the species that produces them (at least in *Clausocalanus* species) so the egg-ratio method (Edmondson, 1971) was rejected for this study. In order to exactly enumerate the number of egg in each sac, the sac was de-

tached from sac-bearing females. Artificial reduction of the time a female carries its clutch of eggs (the egg carrying time) would hence increase clutch turnover rate and reduce the interclutch period (the time between two successive laying events) and consequently, overestimate their egg production rate. Nevertheless, two successive clutch laying events in *Clausocalanus* were only observed in very few occasions so the time used to calculate egg production rates for both broadcast and sac spawner *Clausocalanus* species in this study was the time from the appearance of one clutch to the maximum individual incubation time achieved. I am aware of such overestimated egg production rates but, in the particular case of *Clausocalanus* species, I considered those calculations much more reliable and closer to the real values than those that would be obtained by the traditional 24 h incubation method.

In situ weight-specific fecundity rates measured in females fed on natural particle assemblage were analysed and statistically treated as in Hirst and Bunker (2003) in order to compare the measured rates with their review on growth rates of marine planktonic copepods at global scale. Data were analysed at three different taxonomic levels for better comparison with the literature: the whole genus, species grouped in sac and broadcast spawners, and separate species. Linear regressions between log_e weight-specific fecundity and temperature were used to derive Q_{10} values necessary to correct weight-specific fecundity rates collected at different environmental temperatures to a standard temperature value. The measurement conditions for weight-specific fecundity rates do not account for environmental temperature differences between or in species data points, so descriptive scaling of weight-specific fecundity should not be compensated for them. Compensation is necessary, however, when comparing groups that differ in incubation temperature or searching for uniform explanations for scaling effects that do not

depend on temperature. Rates were standardize to a temperature of 15°C as in previous studies conducted on copepods (e.g. Huntley and Lopez, 1992; Kiørboe and Saiz, 1995; Hirst and Bunker, 2003). An approach that accounts for temperature differences has two further benefits: firstly, it allows for investigation of the influence of body mass on weight-specific fecundity rates without the confounding influence of environmental temperature (it is already known that copepod body size is inversely related to temperature; in Huntley and Lopez, 1992). Secondly, incorporation of temperature into predictive multiple regression models allows for improved estimates of weight-specific fecundity rates when both body mass and environmental temperature are available.

The temperature-corrected rates were then log_{10} transformed and regressed against log_{10} estimated body weight.

Weight-specific fecundity rate relationships with environmental temperature, estimated body weight and Chl *a* concentration were analysed by backward step-wise linear regression (SigmaStat Package, SPSS). The dependent variable was $\log_{10}$ weight-specific fecundity (g, d^{-1}), and independent variables were temperature (T, °C), $\log_{10}$ body weight (BW, μg C ind^{-1}), and $\log_{10}$ Chl *a* concentration (C_a, μg Chl *a* L^{-1}). Backward step-wise linear regression removes the independent variables that do not add significantly to the prediction and the regression is completed using the remaining variables (SigmaStat Package, SPSS). When no independent variables were removed, a multiple linear regression was used:

$$log_{10}\ g = a[T] + b[log_{10}\ BW] + c[log_{10}\ C_a] + d$$

Secondary production and recruitment

Secondary production was estimated according to the "egg production method" (Poulet *et al.*, 1995), which is based on two variables, i.e. E (fecundity of females) and B_f (biomass of females). The secondary production (S) is given by:

$$S = EW_eB_f$$

where E is the egg production (eggs f^{-1} d^{-1}) and B is female abundance (ind. m^{-3}). This is transformed to carbon by multiplying by the estimated egg carbon contents (W_e). This method represents only the fraction of total population secondary production corresponding strictly to the contribution of females (in eggs m^{-3} d^{-1} or in μg C m^{-3} d^{-1}). However, it has some methodological advantages, since fecundity can be easily determined in incubation experiments. Moreover, copepod egg production reflects the integrated response of adult females to environmental conditions prevailing during the accumulation of gonadal material, so it is time-specific and site-specific and provides good spatial and temporal resolution. Nevertheless, it has been shown that adult female growth is not reflected only in egg production but also in increase of its body weight (Hirst and McKinnon, 2001). Lack of information on life-history traits of *Clausocalanus* species, together with difficulties in evaluating life-history in the field were the main reasons for the use of the egg production method to estimate secondary production in this genus in the field for the present study. *Clausocalanus* female abundances from quantitative zooplankton samples and egg production rates measured in the reproduction experiments conducted in parallel to ecological surveys were used to estimate secondary production in *Clausocalanus* species.

Recruitment of naplii stage NI (R, nauplii m^{-3} d^{-1}) could be obtained simply

by multiplying the secondary production (S, in eggs m^{-3} d^{-1}) by V (egg viability or hatching success, in percentage of viable or hatched eggs), becoming:

$$R = EB_f V$$

obtaining the number of recruited nauplii m^{-3} d^{-1} which can also be expressed in carbon units (μg C m^{-3} d^{-1}).

Table 6.1. Stations, incubation conditions, species and parameters studied in the Ionian Sea. Abbreviations: TCB temperature-controlled boxes, TCC temperature-controlled chamber, TCT temperature-controlled thermostat, SST Sea surface temperature, NPA natural particle assemblages, CS clutch size, IP interclutch period, EPR egg production rate, HS hatching success, EV embryo viability, EF egg fertilization.

			Incubation conditions					Studied parameters					
Station	Date	Tow depth (m)	Place	Temp (°C)	Particles suspension	Water	*Species*	CS	IP	EPR	HS	EV	EF
SG99	01/04/2002	50-0	TCB	15	Manually	NPA	*C. lividus*	x		x	x		
I01	04/04/2002	50-0	TCB	15	Manually	NPA	*C. arcuicornis*	x		x	x		
							C. furcatus	x		x	x		
							C. lividus	x		x	x		
							C. mastigophorus	x		x	x		
S07	05/04/2002	50-0	TCB	15	Manually	NPA	*C. arcuicornis*	x		x	x		
							C. furcatus	x		x	x		
							C. lividus	x		x	x		
T05	07/04/2002	50-0	TCB	15	Manually	NPA	*C. furcatus*	x		x	x		
							C. lividus	x		x	x		
							C. mastigophorus	x		x	x		
O04	08/04/2002	50-0	TCB	15	Manually	NPA	*C. arcuicornis*	x		x	x		
							C. furcatus	x		x	x		
							C. jobei	x		x	x		

Table 6.1 (Continued) - Reproduction experiments performed in the Gulf of Naples.

Station	Date	Incubation conditions					Studied parameters					
		Tow depth (m)	Place	Temp (°C)	Particles suspension	Water	*Species*	CS	IP	EPR	HS	EV	EF
MC (521)	27/02/2002	50-0	TCC	16	Plankton wheel	NPA	*C. arcuicornis*	x		x	x		
							C. lividus	x		x	x		
MC (522)	04/03/2002	50-0	TCC	16	Plankton wheel	NPA	*C. arcuicornis*	x		x	x		
							C. furcatus	x		x	x		
							C. lividus	x		x	x		
MC (524)	18/03/2002	50-0	TCC	16	Plankton wheel	NPA	*C. arcuicornis*	x		x	x		
							C. lividus	x		x	x		
L20 2	21/03/2002	50-0	TCC	16	Plankton wheel	NPA	*C. arcuicornis*	x		x	x		
							C. lividus	x		x	x		
							C. parapergens	x		x	x		
L20 3	24/04/2002	50-0	TCC	16	Plankton wheel	NPA	*C. furcatus*	x		x	x		
L20 4	16/05/2002	50-0	TCC	16	Plankton wheel	NPA	*C. arcuicornis*	x		x	x		
							C. furcatus	x		x	x		
							C. lividus	x		x	x		
L20 5	19/06/2002	50-0	TCC	16	Plankton wheel	NPA	*C. furcatus*	x		x	x		
L20 6	03/07/2002	50-0	TCC	20	Plankton wheel	NPA	*C. furcatus*	x		x	x		
L20 7	23/07/2002	50-0	TCC	20	Plankton wheel	NPA	*C. furcatus*	x		x	x		
L20 9	26/08/2002	50-0	TCC	20	Plankton wheel	NPA	*C. furcatus*	x		x	x		
L20 10	03/10/2002	50-0	TCC	20	Plankton wheel	NPA	*C. furcatus*	x		x	x		

Table 6.1 (Continued) - Reproduction experiments performed in the Gulf of Naples. METHOD in Station column refers to those experiments conducted to set the embryo viability method.

		Incubation conditions					Studied parameters					
Station	Date	Tow depth (m)	Place	Temp (ºC)	Particles suspension	Water	Species	CS	IP	EPR	HS	EV	EF
METHOD	Oct-Nov 2003	50-0	TCC	23	Plankton wheel	*Prorocentrum*	*C. furcatus*			x	x	x	
METHOD	April 2004	50-0	TCC	18	Plankton wheel	*Prorocentrum*	*C. furcatus*			x	x	x	
METHOD							*C. arcuicornis*			x	x	x	
MC (708)	03/04/2006	50-0	TCC	16	Plankton wheel	*Prorocentrum*	*C. arcuicornis*	x	x	x	x	x	
							C. jobei	x	x	x	x	x	
							C. lividus	x	x	x	x	x	
							C. pergens	x	x	x	x	x	
MC (709)	10/04/2006	50-0	TCC	16	Plankton wheel	*Prorocentrum*	*C. arcuicornis*	x	x	x	x	x	x
							C. furcatus	x	x	x	x	x	x
							C. pergens	x	x	x	x	x	x
MC (710)	19/04/2006	50-0	TCC	16	Plankton wheel	*Prorocentrum*	*C. arcuicornis*	x	x	x	x	x	x
							C. furcatus	x	x	x	x	x	x
							C. pergens	x	x	x	x	x	x
							C. paululus	x	x	x	x	x	x
MC (711)	26/04/2006	50-0	TCC	16	Plankton wheel	*Prorocentrum*	*C. paululus*	x	x	x	x	x	x
							C. pergens	x	x	x	x	x	x
MC (711)	27/04/2006	50-0	TCC	16	Plankton wheel	*Prorocentrum*	*C. arcuicornis*	x	x	x	x	x	x
							C. furcatus	x	x	x	x	x	x
							C. paululus	x	x	x	x	x	x
							C. pergens	x	x	x	x	x	x
MC (712)	02/05/2006	50-0	TCC	16	Plankton wheel	*Prorocentrum*	*C. arcuicornis*	x	x	x	x	x	x
							C. furcatus	x	x	x	x	x	x
							C. lividus	x	x	x	x	x	x
							C. pergens	x		x	x		

Table 6.1 (Continued) - Reproduction experiments performed in the North-western Mediterranean Sea.

		Incubation conditions					Studied parameters					
Station	Date	Tow depth (m)	Place	Temp (°C)	Particles suspension	Water	Species	CS	IP	EPR	HS	EV	EF
NB4_10	08/03/2003	50-0	TCT	13	Manually	NPA	*C. arcuicornis*	x		x	x		
							C. lividus	x		x	x		
							C. parapergens	x		x	x		
							C. pergens	x		x	x		
NB4_13b	09/03/2003	50-0	TCT	13	Manually	NPA	*C. lividus*	x		x	x		
							C. parapergens	x		x	x		
							C. pergens	x		x	x		
NB4_19	11/03/2003						*C. arcuicornis*	x		x	x		
		50-0	TCT	13	Manually	NPA	*C. pergens*	x		x	x		
NB4_21	11/03/2003						*C. arcuicornis*	x		x	x		
		50-0	TCT	13	Manually	NPA	*C. lividus*	x		x	x		
NB4_28	12/03/2003						*C. arcuicornis*	x		x	x		
		50-0	TCT	13	Manually	NPA	*C. lividus*	x		x	x		
							C. parapergens	x		x	x		
							C. pergens	x		x	x		
NB4_42	17/03/2003	50-0	TCT	13	Manually	NPA	*C. arcuicornis*	x		x	x		
							C. lividus	x		x	x		
							C. pergens	x		x	x		
NB4_68	18/03/2003	50-0	TCT	13	Manually	NPA	*C. arcuicornis*	x		x	x		
							C. pergens	x		x	x		
NB4_89	21/03/2003	50-0	TCT	13	Manually	NPA	*C. arcuicornis*	x		x	x		
							C. pergens	x		x	x		
NB5-04	21/04/2003	50-0	TCT	13	Manually	NPA	*C. arcuicornis*	x		x	x		
							C. lividus	x		x	x		
NB5_22	23/04/2003	50-0	TCT	13	Manually	NPA	*C. arcuicornis*	x		x	x		
							C. lividus	x		x	x		
							C. pergens	x		x	x		

Table 6.1 (Continued) - Reproduction experiments performed in the Atlantic

Area	Station	Date	Tow depth (m)	Incubation conditions				Species	Studied parameters					
				Place	Temp (ºC)	Particles suspension	Water		CS	IP	EPR	HS	EV	EF
NADR	4	21/09/2004	50-0	SST	16.6	Plankton wheel	NPA	*C. arcuicornis*	X		X		X	
								C. pergens	X		X		X	
NADR	6	22/09/2004	50-0	SST	18.7	Plankton wheel	NPA	*C. arcuicornis*	X		X		X	
								C. pergens	X		X		X	
NADR	10	24/09/2004	200-0	SST	21.8	Plankton wheel	NPA	*C. furcatus*	X		X		X	
								C. lividus	X		X	X		
NAST-E	12	25/09/2004	200-0	SST	24.1	Plankton wheel	NPA	*C. furcatus*	X		X		X	
NAST-E	16	27/09/2004	100-0	SST	24.4	Plankton wheel	NPA	*C. furcatus*	X		X		X	
NAST-E	18	29/09/2004	100-0	SST	25.1	Plankton wheel	NPA	*C. furcatus*	X		X		X	
CNRY	23	01/10/2004	100-0	SST	21.5	Plankton wheel	NPA	*C. furcatus*	X		X		X	
								C. mastigophorus	X		X	X		
CNRY	29	03/10/2004	50-0	SST	23.7	Plankton wheel	NPA	*C. furcatus*	X		X		X	
CNRY	30	04/10/2004			26.7	Plankton wheel		*C. jobei*	X		X		X	
CNRY	32	05/10/2004	100-0	SST	20	Manually	NPA	*C. furcatus*	X		X		X	
NATR	38	08/10/2004	100-0	SST	20	Manually	NPA	*C. furcatus*	X		X		X	
WTRA	39	09/10/2004	100-0	SST	20	Manually	NPA	*C. furcatus*	X		X		X	
WTRA	41	10/10/2004	200-0	SST	20	Manually	NPA	*C. furcatus*	X		X		X	
WTRA	44	12/10/2004	100-0	SST	20	Manually	NPA	*C. furcatus*	X		X		X	
WTRA	48	14/10/2004	125-0	SST	24.7	Plankton wheel	NPA	*C. furcatus*	X		X		X	
SATL	50	15/10/2004	150-0	SST	23.8	Plankton wheel	NPA	*C. furcatus*	X		X		X	
SATL	52	16/10/2004	200-0	SST	23.3	Plankton wheel	NPA	*C. furcatus*	X		X		X	
SATL	62	21/10/2004	150-0	SST	18.7	Plankton wheel	NPA	*C. ingens*	X		X	X		
SATL	66	23/10/2004	100-0	SST	17.1	Plankton wheel	NPA	*C. pergens*	X		X		X	
SSTC	68	24/10/2004	200-0	SST	13.8	Plankton wheel	NPA	*C. ingens*	X		X	X		

6.2. Results

6.2.1. Species differences in egg laying features

Among egg-carrying *Clausocalanus* species, different ways of carrying the eggs were observed. *C. pergens*, *C. jobei*, *C. parapergens* and *C. arcuicornis* carry a single sac hanging from the genital pore. These sacs are very fragile and, when they are handled, placed in low volumes of water, or in presence of chemicals, they are detached and spread freely in the water. The only pictures obtained of entire egg sacs were taken from living animals, except in one occasion when the ovigerous female was rapidly fixed in ethyl alcohol. Sac displays different shapes between species: in *C. pergens* the sac resembles a 'ball', in *C. jobei* a 'tear' (Fig. 6.3d), in *C. parapergens* a 'beaver tail' and in *C. arcuicornis* a 'corncob' (Fig. 6.3a,b). *C. paululus* also carries a sac (Frost, 1969) but ovigerous females were not observed during this study despite the incubation of some wild females. Within the sac, the eggs are packed in multiple layers and surrounded by an outer thin membrane that is transparent and becomes whitish and visible only after fixation (Fig. 6.3f). On several occasions and at different sites, when the egg sac of *C. jobei* was dissected, already hatched nauplii appeared in the inner part of the sac (Fig. 6.3e). Such a feature was also observed in *C. arcuicornis* in the Gulf of Naples (spring 2006) when the sac was full of already hatched nauplii. Instead of a hanging sac, *C. furcatus* eggs are directly attached to the urosome in a group where the eggs are disposed in multiple layers and adhere to one another by the outer egg membrane (Fig. 6.3c). In this latter case, the term"egg mass" is used, as for *Pseudocalanus* (Corkett and McLaren, 1978).

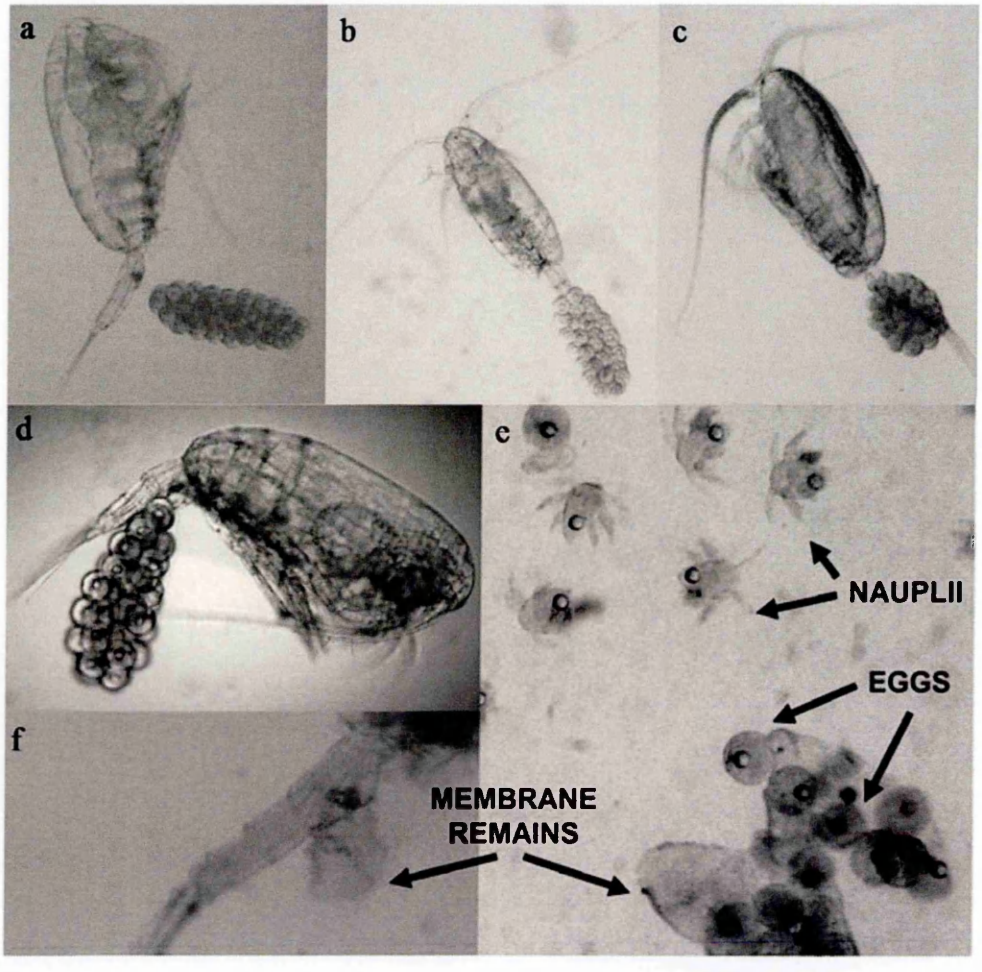

Figure 6.3. Live *C. arcuicornis* ovigerous female in lateral view (a) and ventral view (b); live *C. furcatus* ovigerous female in lateral view (c); fixed *C. jobei* in lateral view (d), dissected sac containing eggs, nauplii and remains of the membrane that surrounded the eggs (e) and detail of the genital pore with sac remains (f).

On some occasions, when the sac was detached from the female, the eggs disintegrated. Similarly, in free spawners the extrusion of shapeless matter released from the genital pore was observed.

Eggs had an oil droplet but they did not float, except on some occasions in the free spawned *C. lividus* and *C. mastigophorus* eggs. Recently hatched nauplii I of *C. furcatus* and *C. jobei* presented oil sacs suggesting that they may not be the first feeding stage (Fig. 6.3c).

6.2.2. Assessment of embryo viability

A methodological protocol for using fluorescent probes on the egg-carrying copepods was set up for *Clausocalanus* (Buttino *et al.*, 2004b) by modifying a previous protocol prepared for the free-spawner copepod *Calanus helgolandicus* (Buttino *et al.*, 2004a). While in *C. helgolandicus* eggs need to be pre-treated with chitinase to allow probes to penetrate thorough the chitinous external membrane, in *C. furcatus* this pre-treatment induced egg disintegration. *C. furcatus* eggs might have a thinner external chitinous membrane, much more permeable than those of most free-spawner copepods. FDA concentration was reduced almost to half that used with *Calanus* obtaining the same fluorescence level (no test was perform to reduce SYTOX green concentration). Time necessary for dye penetration in *C. furcatus* eggs was therefore much shorter (1/4) than in *Calanus* eggs and this shortened the overall procedure (Table 6.2). The new method was first applied to *C. furcatus* and secondly to *C. arcuicornis* (Table 6.3) producing similar results. SYTOX green was used also on eggs of other *Clausocalanus* sac spawners (*C. jobei* and *C. pergens*) as well as on the broadcast spawner *C. lividus* with successful results in all cases.

C. furcatus embryo viability was assessed in wild populations of the Gulf of

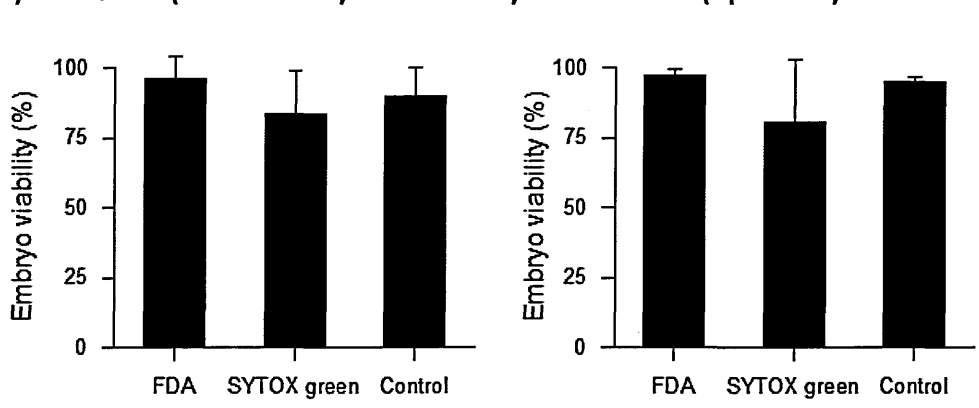

Figure 6.4. Embryo viability assessed with two different vital probes (FDA and SYTOX green) and compared to controls in *C. furcatus* (a), and *C. arcuicornis* (b). Percentage of FDA fluorescent embryos, SYTOX green non-fluorescent embryos and controls were not statistically different (Unpaired t-test; $p<0.001$).

Table 6.2. Dyes protocol: *Clausocalanus* – *Calanus helgolandicus* comparison.

	Clausocalanus (egg-carrying)	*Calanus helgolandicus* Buttino *et al.* (2004b)
Pre-treatment	-	chitinase 1 U/ml
FDA	4 μM	7.5 μM
SYTOX green	20 μM	20 μM
Incubation time	20 min	100 min

Naples in different periods of its seasonal cycle. Data collected during the peak season (autumn) were compared with those collected during the season of lowest abundance (spring)(Table 6.3).

In October, *C. furcatus* embryo viability calculated considering both FDA fluorescent embryos and SYTOX green non-fluorescent embryos was 83.6% ($\pm$ 15.4) and it was not statistically different from that recorded in the controls ($89.9 \pm 10.2\%$; Unpaired t-test; $p<0.001$)(Fig. 6.4a). Similar results were obtained when the same dyes were applied to *C. arcuicornis* in April (Fig. 6.4b). In April, *C. furcatus* em-

Table 6.3. Species and number of embryos (and females) analysed for each fluorescent probe,and periods when the embryo viability was addressed.

Species	Period	FDA	SYTOX	CONTROL
C. furcatus	October 2003	583 (21)	53 (3)	57 (12)
	April 2004	-	56 (2)	63 (3)
C. arcuicornis	April 2004	236 (6)	482 (11)	303 (5)

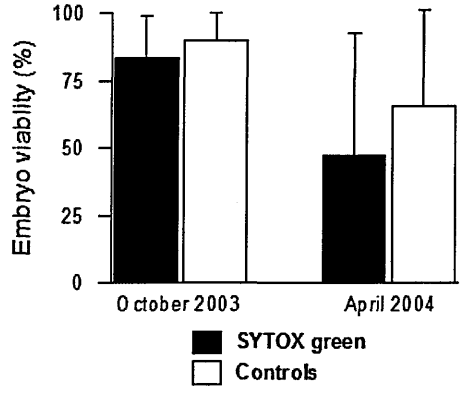

Figure 6.5. *C. furcatus* embryo viability assessed with SYTOX green (black) and controls (white) in two different periods: October 2003 and April 2004. Percentage of SYTOX green non-fluorescent embryos and controls were not statistically different (Unpaired t-test; $p<0.001$) while the two periods were statistically different (Unpaired t-test; $p>0.01$).

bryo viability was 47.2% (± 45.8), significantly different from October (Unpaired t-test; $p>0.05$)(Fig. 6.5). Embryo viability assessed with SYTOX green was not statistically different from that recorded in controls (Unpaired t-test; $p<0.001$).

The effect of detaching the sac from female in *C. arcuicornis* revealed that the control group and the T_7 group had similar embryo viability while the T_0 group presented significant lower embryo viability (Unpaired t-test; $p<0.001$). All three groups presented similar clutch size (Fig. 6.6).

During the experiments with *C. arcuicornis*, several embryos showed partial fluorescence indicating necrosis of part of the embryo. Some of these em-

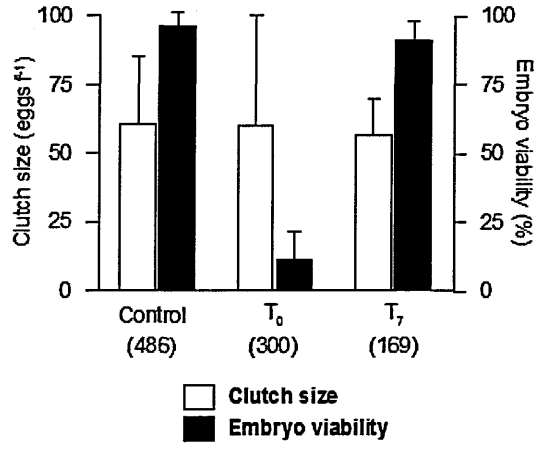

Figure 6.6. *C. arcuicornis* clutch size (white) and embryo viability assessed with SYTOX green (black) in sacs detached at different times: T_0, sacs detached in the first hour of after laying, and T_7, detached after 7-8 h from deposition). The number of embryos observed for each group is indicated in parenthesis.

bryos finally hatched but showed morphological deformation and reduced mobility (Fig. 6.7). Partial embryo fluorescence indicated in some cases deleterious malformation of embryos, while in other cases malformations did not prevent the embryos to reach nauplius I stage.

6.2.3. Reproductive parameters

C. pergens, *C. furcatus*, *C. arcuicornis* and *C. lividus* were studied by analysing more than 50 wild productive females per species in different marine environments and in the laboratory (Table 6.4). The inter-species comparison of reproductive parameters here investigated are summarized in Table 6.5 (clutch size), Table 6.6 (egg production rates), Table 6.7 (embryo viability) and Table 6.8 (interclutch period). Thereafter, the reproductive traits are presented for each species.

Table 6.4. Number of productive *Clausocalanus* females incubated in natural particle assemblages in each surveyed region and in total. In parenthesis are indicated those females incubated in April 2006 under laboratory conditions. per *C. pergens*, fur *C. furcatus*, job *C. jobei*, par *C. parapergens*, arc *C. arcuicornis*, liv *C. lividus*, mas *C. mastigophorus*, and ing *C. ingens*. Lines separate species in three size categories: small, medium and large, respectively.

Species	Ionian Sea Spring 2002	GN Mar-Oct 2002	NW Med. Spring 2003	Atlantic Sep-Oct 2004	TOTAL
C. pergens	-	19 (58)	32	11	62 (58)
C. furcatus	10	121 (4)	-	206	337 (4)
C. jobei	5	(3)	-	5	13 (3)
C. parapergens	-	9	-	-	9
C. arcuicornis	23	47 (20)	40	15	125 (40)
C. lividus	13	26 (8)	28	8	75 (8)
C. mastigophorus	5	-	-	3	8
C. ingens	-	-	-	2	2

Table 6.5. Number of eggs in clutches produced by *Clausocalanus* species incubated in natural particle assemblages at each surveyed region and in total. Mean (range). Lines separate species in three size categories: small, medium and large, respectively.

Species	Ionian Sea	Gulf of Naples	NW Med.	Atlantic	TOTAL
C. pergens	-	17.7 (12-24)	12.5 (3-40)	11.6 (10-15)	14.5 (3-40)
C. furcatus	22.4 (11-50)	20.9 (10-40)	-	17.7 (8-53)	19.0 (8-53)
C. jobei	46.8 (24-75)	34.0 (30-36)	-	38.2 (32-44)	40.5 (24-75)
C. parapergens	-	86.4 (65-119)	-	-	-
C. arcuicornis	49.0 (23-80)	45.8 (25-91)	34.4 (20-80)	35.9 (30-40)	41.6 (20-91)
C. lividus	56.3 (21-110)	117.3 (68-198)	104.8 (20-228)	158.6 (112-185)	105.2 (20-228)
C. mastigophorus	75.0 (56-100)	-	-	80.7 (60-104)	76.9 (56-104)
C. ingens	-	-	-	82.5 (86-93)	-

Table 6.6. Egg production rate (EPR, d^{-1}) of *Clausocalanus* species incubated in natural particle assemblages at each surveyed region and in total. Mean (range). Lines separate species in three size categories: small, medium and large, respectively.

Species	Ionian Sea	Gulf of Naples	NW Med.	Atlantic	TOTAL
C. pergens	-	3.1 (0-12.0)	4.6 (0-30.0)	5.2 (3.0-8.0)	3.9 (0-30.0)
C. furcatus	8.0 (5.5-25.0)	12.9 (8.0-40.0)	-	9.9 (2.4-42.4)	11.2 (2.4-42.4)
C. jobei	14.6 (0-37.5)	10.2 (0-18.0)	-	24.9 (17.3-35.2)	16.3 (0-37.5)
C. parapergens	-	64.8 (0-119.0)	-	-	
C. arcuicornis	19.4 (0-40.0)	18.0 (0-91.0)	20.5 (0-68.0)	19.2 (9.8-32.0)	18.9 (0.-91.0)
C. lividus	9.8 (0-55.0)	46.8 (0-198.0)	18.3 (0-136.7)	59.8 (34.5-80.0)	27.5 (0-198.0)
C. mastigophorus	18.8 (0-50.0)	-	-	30.8 (24-41.6)	21.2 (0-50.0)
C. ingens	-	-	-	59.4 (66.2-52.7)	

Table 6.7. Egg hatching success (%) measured without vital probes (natural) and embryo viability assessed using vital probes (Sytox green or FDA) in *Clausocalanus* species in each surveyed region. Asterisks indicates that in the Atlantic Ocean, *C. lividus* and *C. mastigophorus* embryo viability was assessed without using fluorescent probes (natural). Lines separate species in three size categories: small, medium and large, respectively.

Species	Ionian Sea Natural	Gulf of Naples (SYTOX green)	NW Med. Natural	Atlantic (FDA)
C. pergens	-	99.1 (81.3-100)	82.9 (49.0-100)	99.2 (93.3-100)
C. furcatus	-	85.9 (0-100)	-	84.9 (49.7-100)
C. jobei	-	100	-	98.6 (95.5-100)
C. parapergens	-	-	-	-
C. arcuicornis	-	97.7 (50-100)	-	98.8 (93.8-100)
C. lividus	17 (0-21.4)	100	10.5 (0-30.6)	16.4* (6.9-28.6)
C. mastigophorus	13.9 (4.5-18.0)	-	-	24.1* (19.2-30.0)

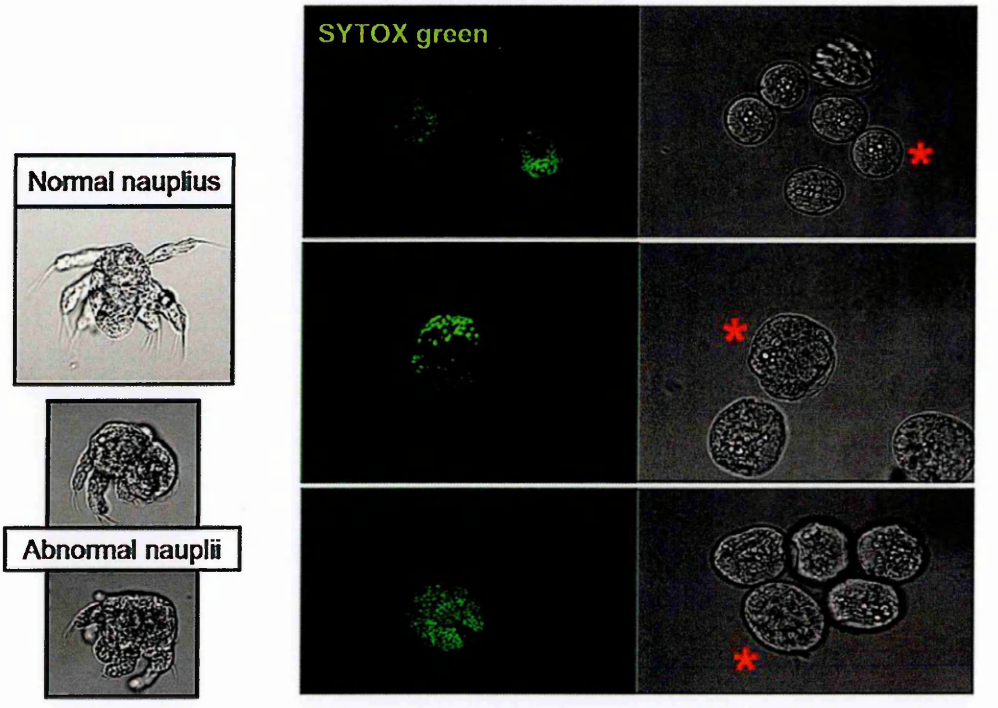

Figure 6.7. Abnormal *C. arcuicornis* embryo and nauplii

Table 6.8. *Clausocalanus* species and areas where interclutch period has been recorded. For the sac spawning *Clausocalanus* species (*C. furcatus*, *C. jobei* and *C. arcuicornis*) interclutch period is underestimated since the sac was detached from the females (see Section 6.1). Lines separate species in three size categories: small, medium and large, respectively.

Species	Area	Interclutch period (h)
C. furcatus	Atlantic Ocean	24-72
C. jobei	Gulf of Naples (2006)	40
C. arcuicornis	Ionian Sea	84
	Gulf of Naples (2006)	48-74
C. lividus	NW Mediterranean	108
	Gulf of Naples (2006)	96

C. pergens (Fig. 6.8, 6.9 it was the smallest sac-spawner species analysed during the present study and was observed in the Gulf of Naples and in the North Balearic Sea (Table 6.4). It presented the smallest clutch size and egg production rate among *Clausocalanus* species. The largest clutch and the highest EPR were recorded in the North Balearic Sea. Embryo viability was very high both in the Gulf of Naples in spring 2006 and in the Atlantic Ocean (Table 6.7). Clutch size, EPR and embryo viability did not vary significantly between *in situ* and laboratory conditions in the Gulf of Naples in spring 2006. Embryo viability decreased under laboratory conditions in successive clutches while the percentage of fertilized eggs was only low in two occasions, one independent of embryo viability and another concomitant to embryo viability decrease (Fig. 6.9 females 9, 10).

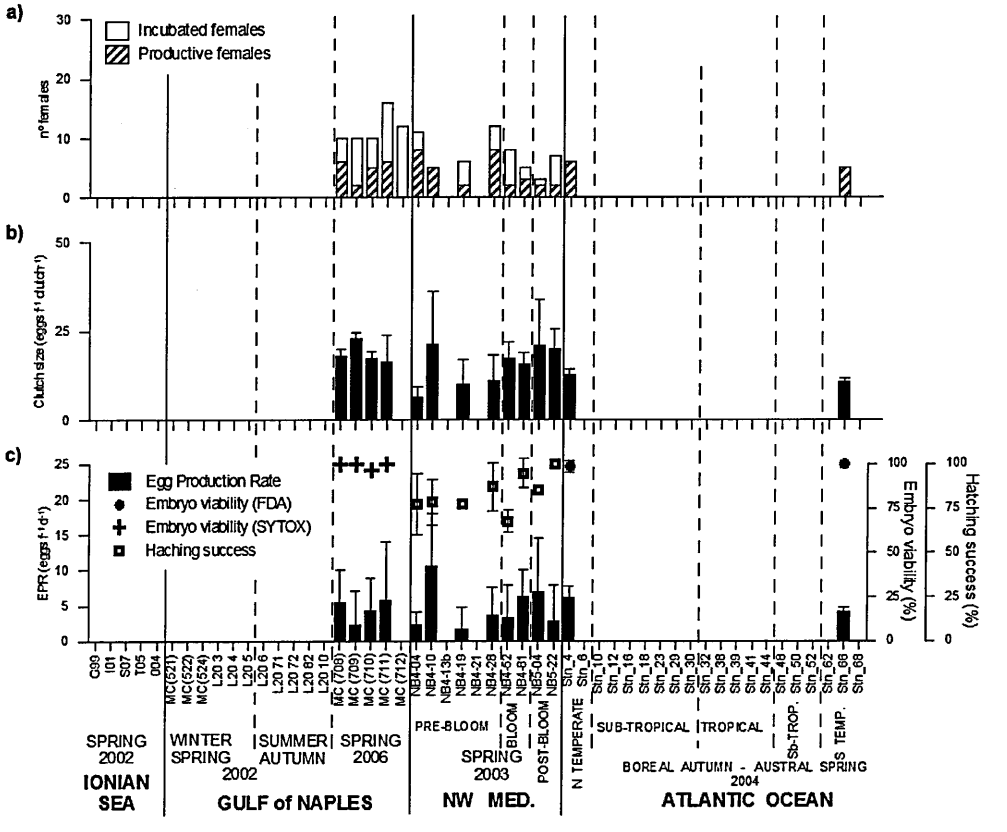

Figure 6.8. *C. pergens.* Number of incubated and productive females (a), mean clutch size (with standard deviation bar)(b), and mean egg production rate with mean embryo viability (each with standard deviation bar)(c) measured in different marine regions, in natural particle assemblages.

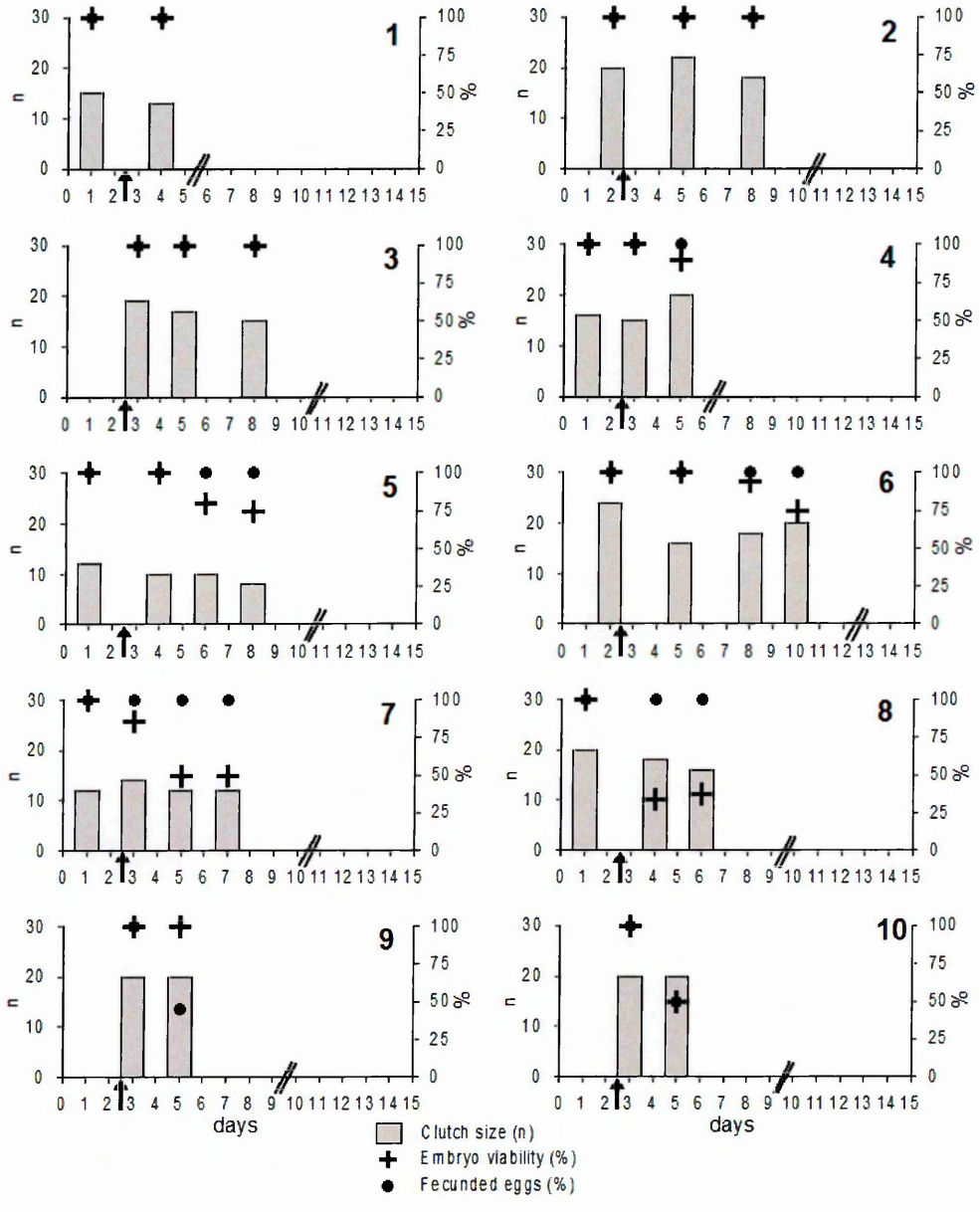

Figure 6.9. *C. pergens* in laboratory experiments. Clutch size (eggs clutch^{-1}), percentage of viable embryos and percentage of fertilized eggs in successive clutches. Arrows indicate 24 h after replace the natural particle assemblage by cultured food; double lines on X axis indicate female death. Individual plots correspond to individual females.

Table 6.9. Reproductive parameters of wild *Clausocalanus* species from the Gulf of Naples in April-May 2006, incubated in two different food conditions: natural particle assemblages and in cultured *Prorocentrum minimum* (30.65 μg C L^{-1}). Mean ± SD. Asterisks indicate significant differences between incubation conditions (one-way ANOVA, $p<0.01$, $p<0.05$). Lines separate species in three size categories: small, medium and large, respectively.

Species	Natural particle assemblages			*Prorocentrum minimum*		
	Clutch size	EPR	Embryo viability	Clutch size	EPR	Embryo viability
C. pergens	17.6 ± 3.2	15.9 ± 3.6	100 ± 0	8.1 ± 2.2	6.1 ± 2.4	84.8 ± 22.4
C. furcatus	22.1 ± 4.2	11.0 ± 2.1	85.8 ± 29.2	26.1 ± 4.8	8.3 ± 4.7	85.2 ± 19.4
C. jobei	34.0 ± 3.5	23.0 ± 11.4	100 ± 0	31.5 ± 2.1	15.6 ± 1.1	100 ± 0
C. arcuicornis	41.1 ± 11.5	14.5 ± 5.2*	97.7 ± 9.9	41.1 ± 13.0	20.5 ± 5.6*	89.5 ± 24.8
C. lividus	71.0 ± 4.2	35.5 ± 2.1*	100 ± 0	72.7 ± 11.5	25.0 ± 5.1*	34.2 ± 49.3

C. furcatus (Fig. 6.10, 6.11) was largely studied in the Gulf of Naples and along the Atlantic meridional transect. Its clutch size was only slightly higher than *C. pergens*, while its EPR was higher. On average, embryo viability was similar in the Gulf of Naples and in the Atlantic Ocean. In the Gulf of Naples, *C. furcatus* clutch size was higher in winter-spring and in summer; in the Atlantic Ocean, it was higher in the NW African upwelling area while it was lower in the northern temperate region (Stn 10 and 12) and in the southern subtropical region (Stn 50 and 52) (one-way ANOVA, $p<0.01$; Fig. 6.10b). EPR was significantly higher in February 2002 (MC-522) and in late July 2002 (L20-72) in the Gulf of Naples and in the upwelling and tropical region in the Atlantic Ocean (Stn 23, 29) but was lower in May 2006 in the Gulf of Naples (GN-06.5) and in the northern temperate (Stn 10) and southern subtropical (Stn 50 and 52) in the Atlantic Ocean (Fig. 6.10c). *C. furcatus* embryo viability in the Atlantic was on average 84.9% ($\pm$ 14.4), significantly higher in the northern sub-tropical region (one-way ANOVA, $p<0.01$) and significantly lower in the southern sub-tropical region (one-way ANOVA, $p<0.01$). Observed interclutch period ranged from 24 to 72 hours. No differences were observed in reproductive parameters between incubation conditions in April 2006 in the Gulf of Naples (Table 6.9). Embryo viability decreased at the fourth and fifth observed clutches while eggs were always fertilized.

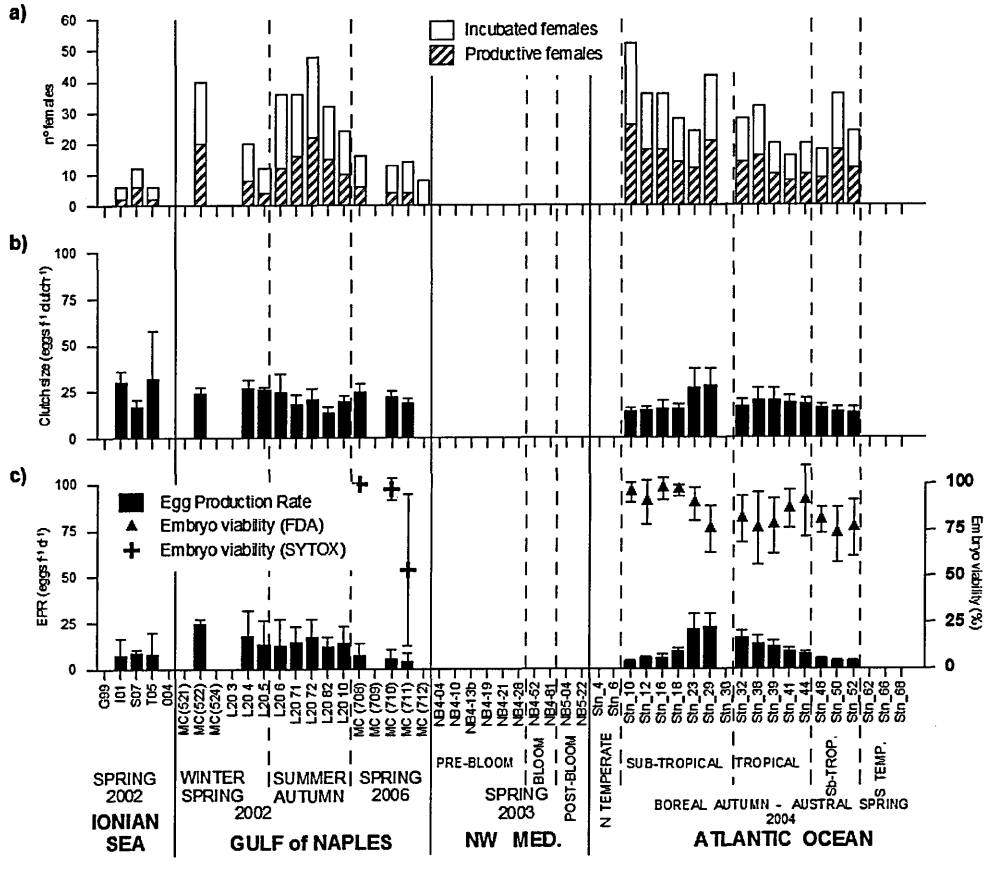

Figure 6.10. *C. furcatus.* Number of incubated and productive females (a), mean clutch size (with standard deviation bar)(b), and mean egg production rate with mean embryo viability (each with standard deviation bar)(c) measured in different marine regions, in natural particle assemblages.

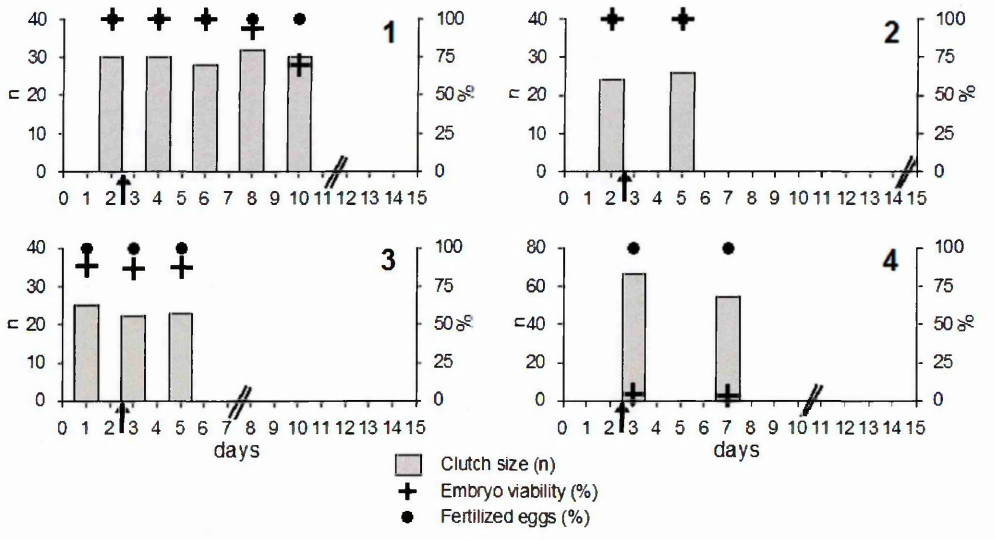

Figure 6.11. *C. furcatus* (females 1 to 3) and *C. lividus* (female 4) laboratory experiments. Clutch size (eggs clutch^{-1}), percentage of viable embryos and percentage of fertilized eggs in successive clutches. Arrows indicate 24 h after replace the natural particle assemblage by cultured food; double lines on X axis indicate female death. Individual plots correspond to individual females.

C. jobei (Fig. 6.12) Reproductive parameters in the medium-sized *C. jobei* were collected in very few females. On average, clutch size was much larger than in the small *C. pergens* and *C. furcatus* while the egg production rates were in the similar range of *C. furcatus*. Embryo viability was very high. No statistical differences were observed among areas either in clutch size, egg production rates or embryo viability. The observed interclutch period was 40 h. In spring 2006 in the Gulf of Naples, no statistical differences were observed in any of the studied parameters. In spring 2006 in the Gulf of Naples, no statistical differences between incubation conditions were observed in reproductive parameters (Table 6.9).

C. parapergens (Fig. 6.12) Reproductive parameters were recorded only in 9 females in the Gulf of Naples. Mean clutch size was very large in comparison with its similar in size species *C. jobei* and *C. arcuicornis*, while the largest clutch (119 eggs) was in the range of the broadcast spawners *C. lividus* and *C. mastigophorus*.

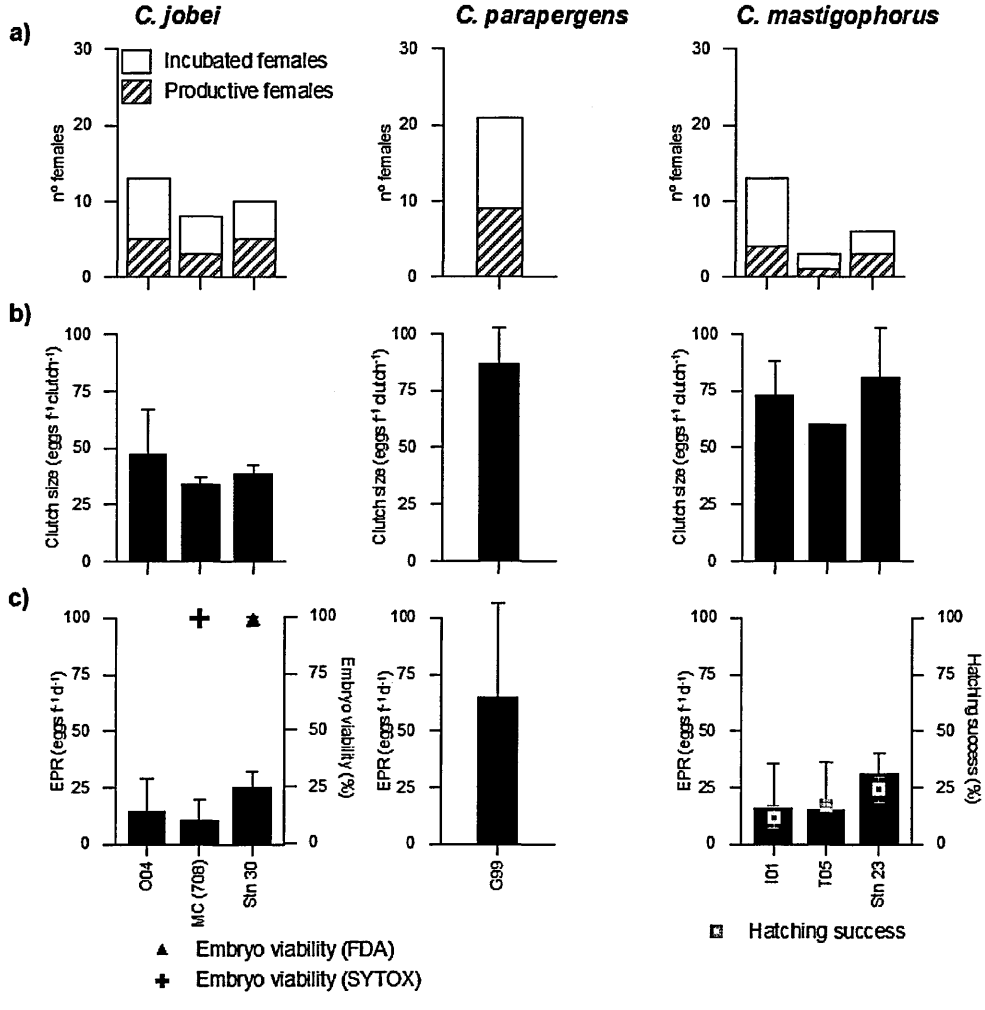

Figure 6.12. *C. jobei*, *C. mastigophorus* and *C. parapergens*. Number of incubated and productive females (a), mean clutch size (with standard deviation bar)(b), and mean egg production rate with mean embryo viability (each with standard deviation bar)(c) measured in different marine regions, in natural particle assemblages.

Mean egg production rate was very high, probably due to short time incubation (24 h) and absence of non productive females.

C. arcuicornis (Fig. 6.13) It was one of the most studied species (Table 6.4, Fig. 6.13). It had similar clutch size and slightly higher egg production rate than the similarly sized congeneric species *C. jobei*. Its embryo viability was very high. Clutch size and EPR showed both significant higher values in May 2002 in the Gulf of Naples and only EPR in the NW Mediterranean (one-way ANOVA, $p<0.01$), while embryo viability did not present significant differences among areas. Interclutch period was on average 48 h became longer at the end of female life (Fig. 6.14 7-10), the largest interval was observed in the Ionian Sea. EPR observed in spring 2006 in the Gulf of Naples showed statistically different values under different incubation conditions. Only two wild females did not show 100% embryo viability under natural particle assemblages (50 and 86.7%). Both females fed on cultured food layed a second clutch after one or two days showing opposite responses: the one with the lowest embryo viability layed a second clutch with higher embryo viability (88.2%) while the other female layed a second clutch with lower embryo viability (75%). In some cases, embryo viability gradually decreased among clutches without a parallel decrease in percentage of fertilized eggs (Fig. 6.14 females 5, 7, 9), while in other cases embryo viability slightly or suddently dropped at the last clutch in correspondance with a decrease in percentage of fertilized eggs (Fig. 6.14 females 6, 8, 10).

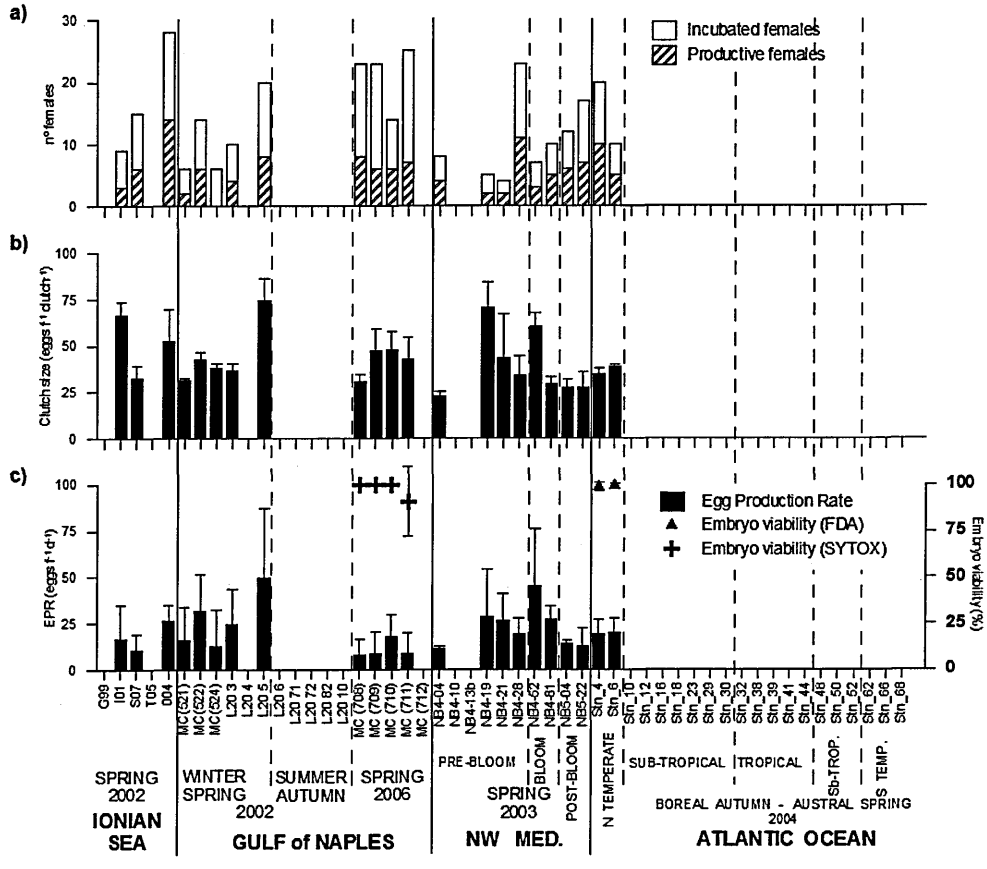

Figure 6.13. *C. arcuicornis.* Number of incubated and productive females (a), mean clutch size (with standard deviation bar)(b), and mean egg production rate with mean embryo viability (each with standard deviation bar)(c) measured in different marine regions, in natural particle assemblages.

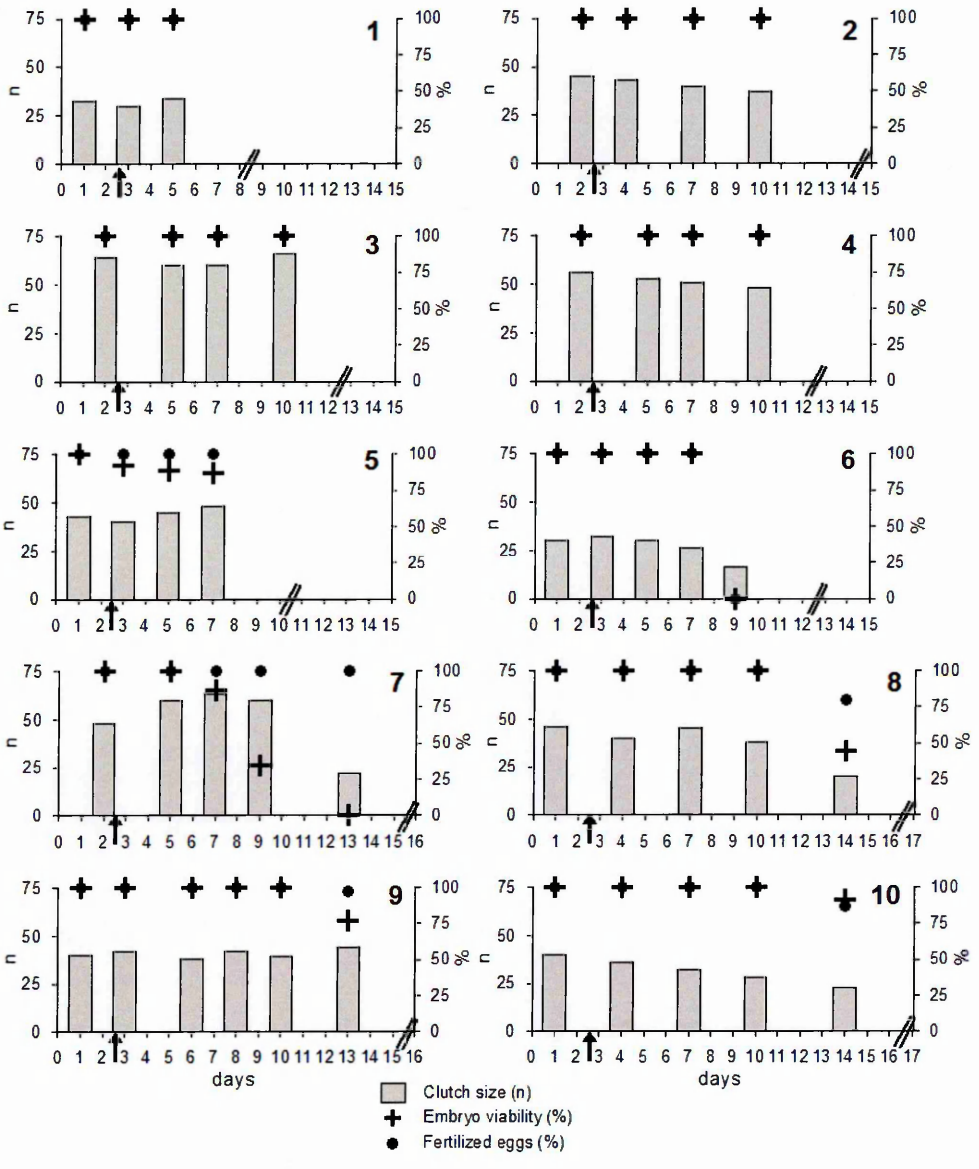

Figure 6.14. *C. arcuicornis* laboratory experiments. Clutch size (eggs clutch^{-1}), percentage of viable embryos and percentage of fertilized eggs in successive clutches. Arrows indicate 24 h after replace the natural particle assemblage by cultured food; double lines on X axis indicate female death. Individual plots correspond to individual females.

C. lividus (Fig. 6.15 On average, clutch size and EPR were higher than in sac-spawner congeneric species, while the embryo viability was the lowest observed in *Clausocalanus*. Clutch size was statistically lower in the Ionian Sea, while EPR was statistically higher in the Gulf of Naples in winter-spring 2002 (one-way ANOVA, $p<0.05$; Fig. 6.15 b). Interclutch period of *C. lividus* was the longest among *Clausocalanus* species (Table 6.8). Under different incubation conditions, clutch size did not differed significantly, while EPR was lower when incubated in dinoflagellate culture ($p<0.05$). In several occasions, observed embryo viability was zero, while egg fertilization was 100% also in two successive clutches like their feeding history were compromising their brood (Fig. 6.11).

C. mastigophorus (Fig. 6.12) Reproductive parameters were measured only in 8 *C. mastigophorus* females. On average, clutch size and EPR were similar to *C. lividus* and larger than the sac-spawner congeners. Embryo viability was very low, as *C. lividus*. Clutch size, egg production rate and embryo viability were not different in the two areas where reproductive parameters of this species were measured (one-way ANOVA, $p>0.05$).

C. ingens Only two females of *C. ingens* were observed (Table 6.4). Its clutch size and egg production were on the range of the other broadcast spawners *C. lividus* and *C. mastigophorus*.

A summary of *Clausocalanus* species reproductive parameters is shown in Table 6.10.

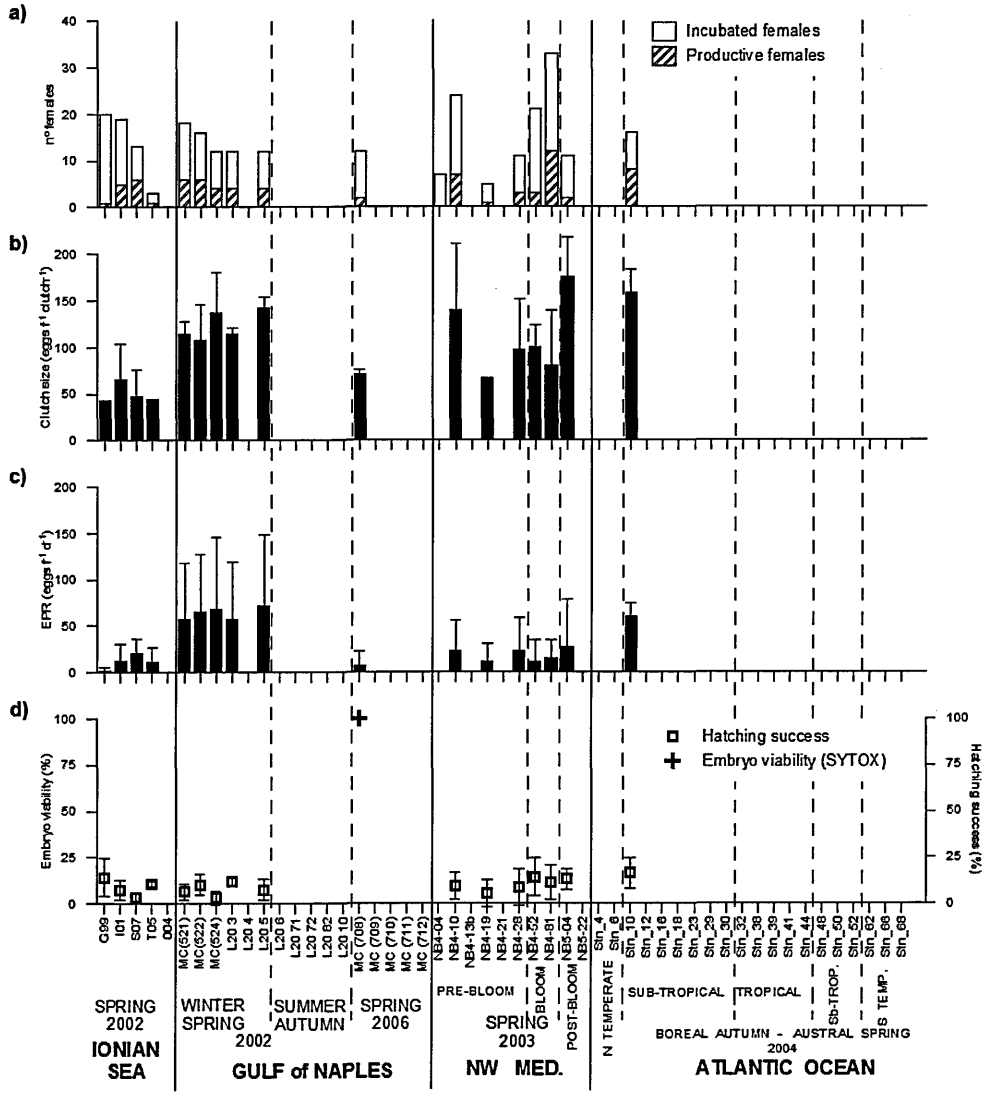

Figure 6.15. *C. lividus*. Number of incubated and productive females (a), mean clutch size (with standard deviation bar)(b) and mean egg production rate and mean embryo viability (each with standard deviation bar)(c) measured at different natural conditions.

Table 6.10. *Clausocalanus* species reproductive parameters: number of eggs in clutches (clutch size), egg production rate (EPR, d^{-1}), hatching success (%), and embryo viability (%) assessed with FDA or Sytox green (vital probes). Mean (SD). Lines separate species in three size categories: small, medium and large, respectively.

Species	Clutch size	EPR	Hatching success	FDA	Sytox green
C. pergens	14.5 (7.4)	3.9 (5.2)	84.1 (14.2)	99.2 (2.4)	99.1 (4.1)
C. furcatus	19.0 (7.1)	13.5 (8.6)	82.9 (13.4)	84.9 (14.4)	85.9 (29.2)
C. jobei	40.5 (13.1)	22.5 (8.0)		98.6 (2.1)	100.0 (0.0)
C. parapergens	65.1 (25.3)	44.0 (29.1)			
C. arcuicornis	41.6 (16.2)	27.4 (16.6)	95.5 (3.4)	98.8 (2.1)	97.7 (9.9)
C. ingens	82.5 (5.0)	59.4 (9.5)	78.8 (6.5)		
C. lividus	105.2 (51.8)	67.8 (45.2)	9.6 (7.3)		100.0 (0.0)
C. mastigophorus	76.9 (18.2)	35.3 (9.2)	17.3 (6.9)		

Weight-specific fecundity

Measures of females prosome length and egg diameter used to estimate species weight-specific fecundity are reported in Table 6.11 and 6.13. Measures of females total length are also reported in Table 6.12.

Clutch weight represented less than 25% of the body weight in *Clausocalanus* sac spawners and 25-75% in broadcast species (Fig. 6.16).

Estimated weight-specific fecundity rates in *Clausocalanus* species ranged from 0.01 to 0.77 d^{-1}, being higher in broadcast spawners than in sac spawners. The only exception was represented by *C. parapergens* in spring 2002 in the Gulf of Naples (0.225 ± 0.042 d^{-1})(Table 6.14, Fig. 6.17).

Regression of estimated weight-specific fecundity rates versus temperature revealed a significant decrease of fecundity with increase in temperature, mainly driven by sac-spawning species ($p<0.001$ both at the genus level and in the sac-spawning group)(Table 6.15). In broadcast *Clausocalanus*, weight-specific fecundity did not show significant relationship with temperature ($p>0.1$). However, at the species level, differentiated relationships were observed. In *C. arcuicornis* and *C. lividus*, weight-specific fecundity increased significantly with temperature ($p<0.001$ and $p<0.1$, respectively) while in *C. furcatus* it decreased significantly ($p<0.001$). In *C. jobei*, *C. pergens* and *C. mastigophorus*, no significant relationship with temperature was observed (Table 6.15; Fig. 6.18a).

Weight-specific fecundity scaled positively with body weight at genus level ($p<0.001$) and for sac-spawners ($p<0.001$) and negatively ($p<0.001$) for broadcast spawners (Table 6.16; Fig. 6.18b).

Michaelis-Menten plots for the weight-specific fecundity rates corrected at 15°C did not fit significantly with *Clausocalanus* data either at genus, group (broadcast/sac-spawners) or species level (Fig. 6.18c).

Table 6.11. *Clausocalanus* females prosome length (mean and range, expressed in μm) at each studied area and estimated carbon contents (μg C) used to estimate weight-specific fecundity rates.

Spp	Ionian Sea	Gulf of Naples		NW Mediter.	Atlantic Ocean		
	Spring 2002	Spring 2002	Summer 2002	Spring 2003	North Atlantic	CNRY region	Tropical region
per	-	663.6	-	749.8	613.1	-	-
		(632-726.8)		(663.6-850)	(571.5-666.8)		
		2.4		3.6	1.9		
		(2.0-3.2)		(2.4-5.2)	(1.5-2.4)		
fur	833.8	824.3	732.9	-	-	744.8	784.1
	(800-937.5)	(800-850)	(700-750)			(700-790)	(730-850)
	4.9	4.7	3.2			3.4	4.0
	(4.3-7.0)	(4.3-5.2)	(2.8-3.5)			(2.8-4.1)	(3.2-5.2)
job	870.0	867.5	-	-	-	956.3	-
	(800-910)	(860-880)				(875-1000)	
	5.6	5.5				7.5	
	(4.3-6.4)	(5.3-5.7)				(5.6-8.6)	
par	-	925.0	-	-	-	-	-
		(900-950)					
		6.7					
		(6.2-7.3)					
arc	772.7	934.7	-	906.7	879.0	-	-
	(750-800)	(830-1000)		(850-950)	(812.5-987.5)		
	3.8	7.0		6.3	5.8		
	(3.5-4.3)	(4.8-8.6)		(5.2-7.3)	(4.5-8.2)		
liv	1161.7	1204.2	-	1465.6	1078.4	-	-
	(1125-1200)	(1109.4-1281.3)		(1312.5-1546.9)	(1025-1125)		
	13.7	15.0		28.7	10.9		
	(12.4-15.2)	(11.9-18.6)		(20.1-33.5)	(9.3-12.4)		
mas	1171.4	-	-	-	1062.5	1308.0	-
	(1100-1212.5)				(1050-1075)	(1250-1359.4)	
	14.1				10.4	19.9	
	(11.5-15.7)				(10.0-10.7)	(17.2-22.4)	

Table 6.12. *Clausocalanus* females total length (mean and range, expressed in μm) at each studied area compared to those measures reported by Frost and Fleminger (1968).

Spp	Ionian Sea	Gulf of Naples		NW Mediter.	Atlantic Ocean			F&F-1968
	Spring 2002	Spring 2002	Summer 2002	Spring 2003	North Atlantic	CNRY region	Tropical region	
per	-	882.2	-	965.0	826.5	-	-	860
		845.3-948		870-1070	774.7-889			700-1100
fur	1063.8	1048.6	961.4	-	967.6	-	1015.2	1090
	1010-1187.5	1020-1080	920-990		920-1020		970-1080	940-1310
job	1216.0	1216.3	-	-	-	1322.5	-	1210
	1130-1260	1210-1240				1162.5-1437.5		1010-1560
par	1179.7	1173.8	-	-	-	-	-	1170
	1125-1250	1150-1200						970-1380
arc	982.7	1162.4	-	1113.3	1170.5	-	-	1320
	950-1020	1050-1220		1050-1160	1100-1325			1150-1620
liv	1524.2	1550.0	-	1814.6	1438.0	-	-	1520
	1450-1575	1468.75-1609.4		1640.6-1906.3	1337.5-1512.5			1260-1770
mas	1548.2	-	-	-	1385.7	1692.0	-	1560
	1475-1600				1362.5-1412.5	1625-1750		1230-1840

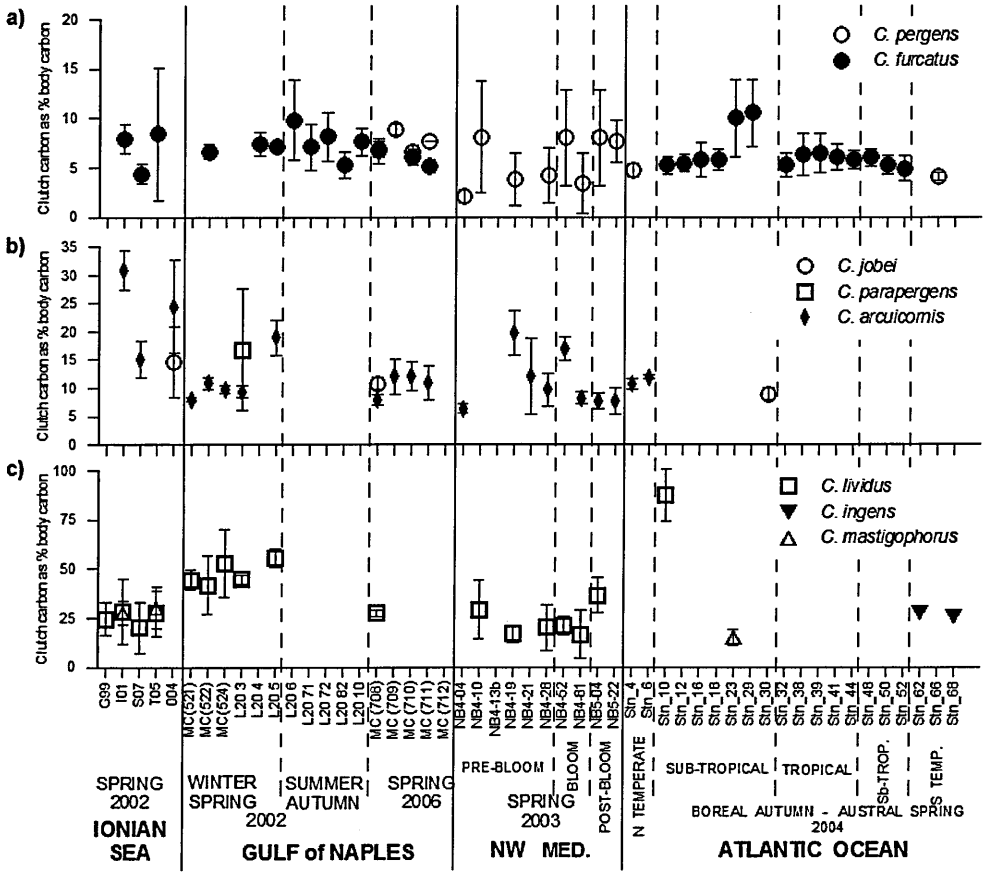

Figure 6.16. Weight-specific reproductive index, i.e. the percentage that the clutch represents on female body weight. (a) *C. pergens* and *C. furcatus* (small-sized species, sac spawners), (b) *C. parapergens*, *C. jobei* and *C. arcuicornis* (medium-sized species, sac spawners), (c) *C. lividus*, *C. ingens* and *C. mastigophorus* (large-sized species, broadcasters).

Table 6.13. Species eggs diameter sporadically measured at the inverted confocal laser scanning microscope (CLSM-Zeiss 410). Those with an asterisk were measured at the stereoscope. Lines separate species in three size categories: small, medium and large, respectively.

Species	Egg diameter (μm)
C. pergens	50*
C. furcatus	56
C. jobei	62
C. parapergens	62*
C. arcuicornis	62.5
C. lividus	93.5
C. mastigophorus	90*
C. ingens	90*

Backward step-wise multiple linear regression of $\log_{10}$ weight-specific fecundity rates at genus level versus temperature, $\log_{10}$ Chl *a* and $\log_{10}$ body weight, revealed that the combination of the three parameters improved the model. The same was observed in sac-spawning species but not in broadcast spawners, in which either Chl *a* or temperature did not improve the significance of the model (Table 6.17). At the species level, temperature in *C. arcuicornis* and *C. pergens*, and Chl *a* in *C. furcatus* did not improved the significance of the model. In *C. jobei*, Chl *a* improved the significance of the model but temperature was constant and not included in the model. *C. parapergens*, *C. ingens* and *C. mastigophorus* were examined only at a single station and were not included in the model.

Measured weight-specific fecundity rates were compared to rates predicted from the models of Ikeda and Motoda (1978), Huntley and Lopez (1992), Hirst and Lampitt (1998), Peterson *et al.* (2002) and Hirst and Bunker (2003)(Table 6.18, Fig. 6.19). If agreement between measured and predicted values was good, most of the data points would fall along a line with slope equal to 1. The Ikeda and Motoda model produced rates similar to those of *Clausocalanus* broadcast

Table 6.14. Estimated weight-specific fecundity rates (g, d^{-1}) of *Clausocalanus* species. Lines separate species in three size categories: small, medium and large, respectively.

Species	Mean WSF (SD)
Genus	0.079 ($\pm$0.099)
Sac spawners	0.055 ($\pm$0.045)
Broadcast spawners	0.229 ($\pm$0.178)
C. pergens	0.022 ($\pm$0.149)
C. furcatus	0.046 ($\pm$0.031)
C. jobei	0.058 ($\pm$0.027)
C. parapergens	0.225 ($\pm$0.042)
C. arcuicornis	0.082 ($\pm$0.048)
C. lividus	0.242 ($\pm$0.186)
C. mastigophorus	0.122 ($\pm$0.042)
C. ingens	0.194 ($\pm$0.031)

spawners < 0.2 day^{-1} but overestimated weight-specific fecundity rates in sac-spawning *Clausocalanus*. The Huntley and Lopez model both overestimates and underestimates with respect data observed in broadcast spawners, while it only overestimates data in sac-spawners. The Hirst and Lampitt, as well as Hirst and Bunker models produce rates similar to those values measured in *Clausocalanus* for g <0.1 d^{-1} while underestimate when g > 0.1 d^{-1}. The Peterson et al. models produce the lowest predicted rates (Fig. 6.19).

Table 6.15. Relationships between log_e weight-specific fecundity rates (g, d^{-1}) versus temperature (T, °C), *see* Fig. 6.18a. Q_{10} correction factor derived from the slope as $Q_{10} = e^{(10*slope)}$. Lines separate species in three size categories: small, medium and large, respectively.

		Temperature		Pearson Correlation						
	N	Mean	SD		sign.	Interception	Slope	r^2	p	Q_{10}
Genus	638	18.53	4.43	-0.211	<0.001	-2.170	-0.046	0.045	<0.001	0.63
Sac spawners	549	19.05	4.46	-0.126	0.002	-2.770	-0.023	0.016	0.001	0.79
Broadcast spawners	89	15.36	2.60	0.34	0.001	-3.650	0.119	0.116	0.003	3.29
Sac spawners										
C. pergens	63	14.29	1.64	0.121	0.172	-4.886	0.056	0.121	0.343	1.75
C. furcatus	337	21.74	3.42	-0.141	0.005	-2.669	-0.030	0.141	0.01	0.74
C. jobei	13	16.63	2.91	0.164	0.297	-3.444	0.029	0.164	0.593	1.34
C. parapergens	9	15.35	-	-	-	-	-	-	-	-
C. arcuicornis	127	14.80	1.67	0.342	<0.001	-4.406	0.118	0.342	<0.001	3.25
Free spawners										
C. lividus	78	15.16	2.50	0.445	<0.001	-4.374	0.170	0.445	<0.001	5.47
C. mastigophorus	9	16.95	3.09	-0.749	0.01	-0.670	-0.088	0.749	0.02	0.41
C. ingens	2	16.01	3.48	-	-	-2.390	0.046	(1)	-	1.58

Table 6.16. Relationships between log_{10} weight-specific fecundity (g, d^{-1}) corrected at 15°C versus log_{10} body weight (BW, μg C ind^{-1}), *see Fig. 6.18b*. Lines separate species in three size categories: small, medium and large, respectively.

Group	N	Body weight Mean	Body weight SD	Pearson Correlation	sign.	Interception	Slope	r^2	p
Genus	638	6.34	5.89	0.556	<0.001	-2.08	0.998	0.344	<0.001
Sac spawners	549	4.27	1.38	0.392	<0.001	-2.132	1.141	0.172	<0.001
Broadcast spawners	89	19.14	6.88	-0.583	<0.001	1.668	-1.945	0.404	<0.001
Sac spawners									
C. pergens	63	2.89	0.7	-0.247	0.025	-1.453	-0.758	0.065	0.044
C. furcatus	337	3.7	0.53	-0.042	0.221	-1.519	-0.014	0	0.964
C. jobei	13	6.3	0.98	0.227	0.228	-2.142	1.104	0.095	0.305
C. arcuicornis	127	6.07	1.13	-0.256	0.002	-0.600	-0.731	0.056	0.007
Broadcast spawners									
C. lividus	78	19.56	7.19	-0.626	<0.001	2.156	-2.314	0.479	<0.001
C. mastigophorus	9	16.05	2.89	-0.858	<0.001	2.820	-3.195	0.845	<0.001

Table 6.17. Backward stepwise regression. Relationships between the dependent log_{10} weight-specific fecundity (g, d^{-1}) versus the independent variables temperature (T, °C), log_{10} body weight (BW, μg C ind^{-1}) and Chl a concentration (Chl a; μg Chl a L^{-1}). Multiple linear regression: log_{10} g = a(T) + b(BW) + c(Chl a) + d. Variable in = Variable introduced, variable out = variable removed. Lines separate species in three size categories: small, medium and large, respectively.

Group	N	Backward stepwise regression	T a	BM b	Chl a c	ct d	r^2	model p	T p	BW p	Chl a p
Genus	579	All in	-0.016	0.943	-0.054	-1.797	0.373	<0.001	<0.001	<0.001	0.076
Sac spawners	511	All in	-0.001	1.155	0.006	-2.127	0.449	<0.001	0.729	<0.001	0.836
		Chl a out	-0.002	1.155	-	-2.122	0.449	<0.001	0.577	<0.001	-
		T out	-	1.183	-	-2.176	0.448	<0.001	-	<0.001	-
Broadcast spawners	68	All in	0.007	-2.197	-0.177	1.946	0.840	<0.001	0.658	<0.001	0.025
		T out	-	-2.301	-0.176	2.194	0.839	<0.001	-	<0.001	0.025
Sac spawners											
C. pergens	63	All in	0.058	-0.806	0.402	-2.22	0.305	<0.001	0.276	0.292	<0.001
		BW out	0.108		0.398	-3.287	0.292	<0.001	<0.001	-	<0.001
C. furcatus	327	All in	-0.031	-1.175	0.144	-0.059	0.150	<0.001	<0.001	<0.001	<0.001
C. jobei	8	Only Chl a	-	-	-0.709	-1.75	0.545	0.037	-	-	0.037
C. arcuicornis	104	All in	0.143	3.412	0.181	-6.085	0.502	<0.001	<0.001	<0.001	<0.001
Broadcast spawners											
C. lividus	63	All in	-0.022	-2.292	-0.192	1.856	0.754	<0.001	0.332	<0.001	0.029
		T out	-	-2.561	-0.206	2.528	0.750	<0.001	-	<0.001	0.018

Table 6.18. Equations used to compare measured weight-specific fecundity rates in *Clausocalanus* from published empirical models as a function of temperature (T, °C), body weight (BW, μg C ind^{-1}) or dry body weight (DBW, mg C ind^{-1}), and Chl *a* (Chl *a*, μg Chl *a* L^{-1}).

Group	Equation	r^2	n	Authors
Zooplankton	$g=(7.714*10^{0.02538T-0.1259}*DBW^{-0.01089T-0.8918})/BW$	-	-	Ikeda and Motoda (1978)
Sac and free spawners	$g=0.0445e^{0.111T}$	0.910	181	Huntley and Lopez (1992)
Sac spawners	$\log_{10}g=-1.7726+0.0385T$	0.311	-	Hirst and Lampitt (1998)
	$\log_{10}g=-1.9869-0.0512T+0.0298Chl$	0.051	88	Peterson et al. (2002)
	$\log_{10}g=0.0182T+0.193\log_{10}BW+0.195\log_{10}Chl-1.591$	0.113	320	Hirst and Bunker (2003)
Free spawners	$\log_{10}g=-0.6516-0.5244\log_{10}BW$	0.435	-	Hirst and Lampitt (1998)
	$\log_{10}g=-0.6286+0.0468T-0.0528Chl+0.0214\log_{10}(BW*T)$	0.048	276	Peterson et al. (2002)
	$\log_{10}g=0.0125T-0.230\log_{10}BW+0.729\log_{10}Chl-1.348$	0.357	1639	Hirst and Bunker (2003)

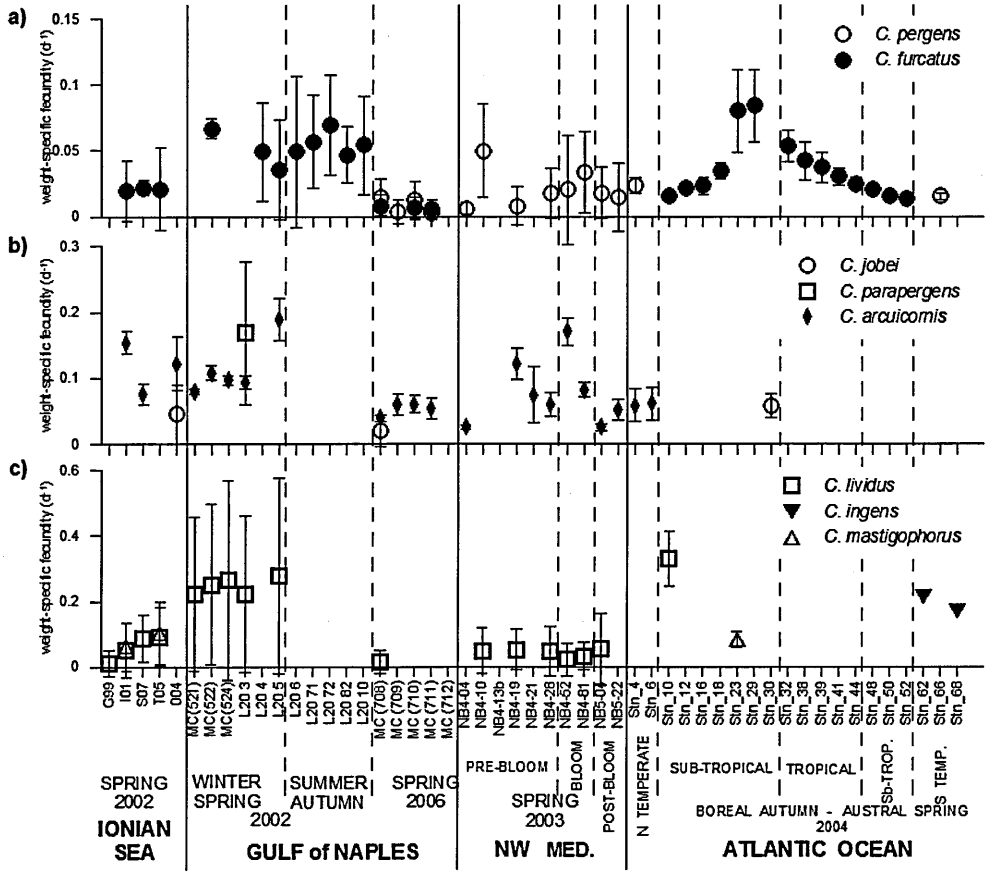

Figure 6.17. Estimated weight-specific fecundity rates of *Clausocalanus* species in different regions. (a) *C. pergens* and *C. furcatus* (small-sized species, sac spawners), (b) *C. parapergens*, *C. jobei* and *C. arcuicornis* (medium-sized species, sac spawners), (c) *C. lividus*, *C. ingens* and *C. mastigophorus* (large-sized species, broadcasters).

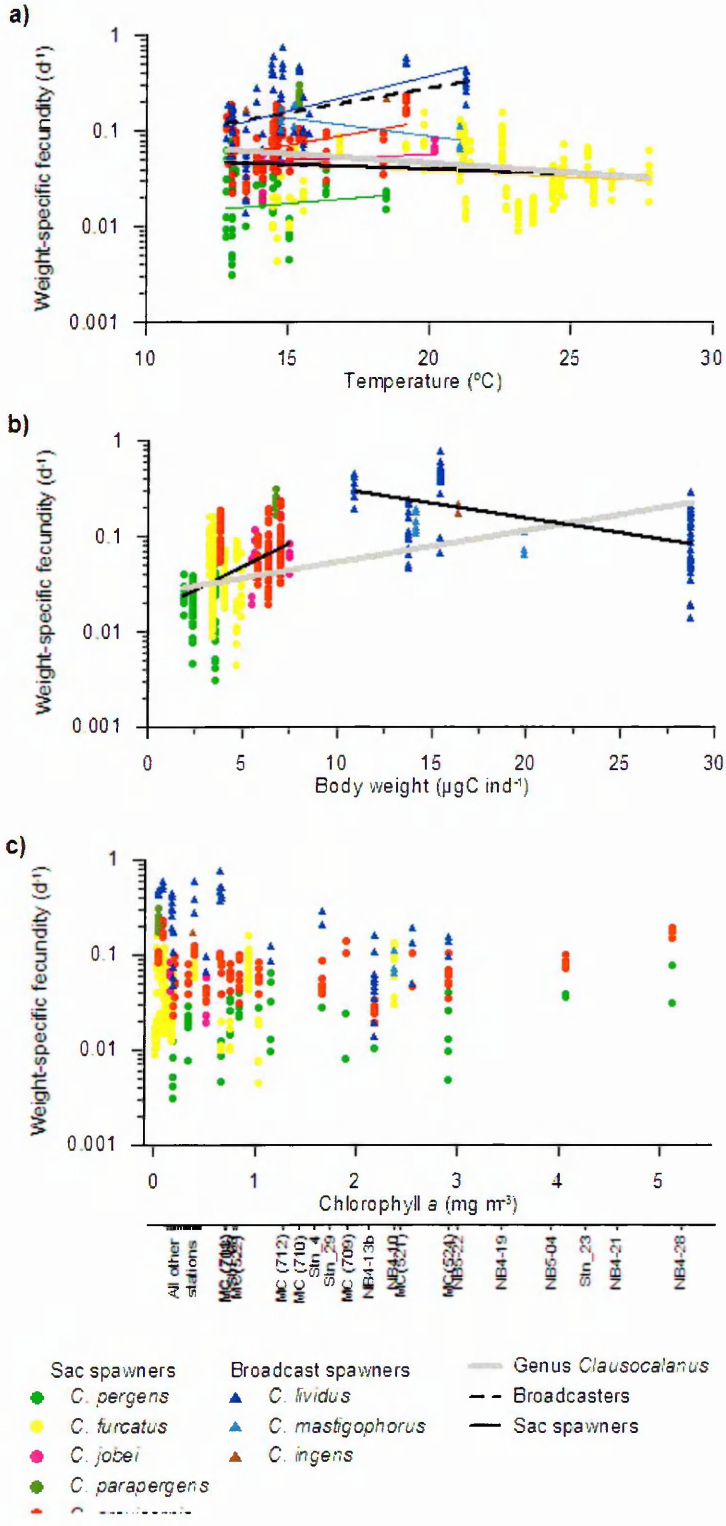

Figure 6.18. *Clausocalanus* weight-specific fecundity rates (WSF) versus temperature (a); WSF rates corrected at 15°C versus estimated body weight (b); and WSF rates corrected at 15°C and 10 μg C ind^{-1} versus Chl *a* concentration (c). Lines describe regressions.

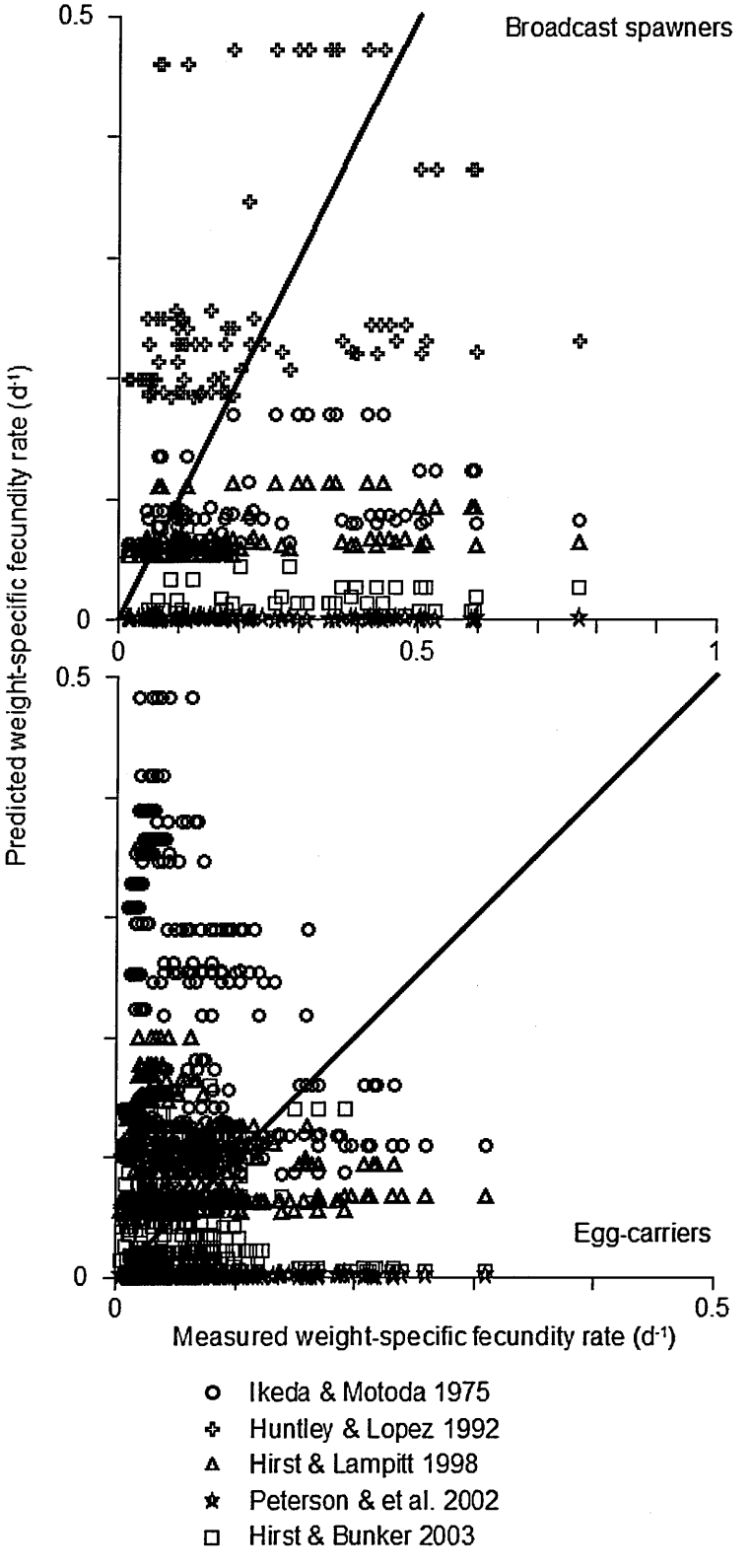

Figure 6.19. Individual weight-specific fecundity rates measured for *Clausocalanus* compared to those predicted rates from models (equations and references of the models are reported in Table 6.18). Solid line has a slope equal to 1.

6.2.4. Secondary production

Secondary production in *C. pergens* did not show significant difference among regions (Fig. 6.20a). *C. furcatus* secondary production was significantly higher in the tropical Atlantic region ($p<0.001$)(Fig. 6.20a). The medium-sized *C. jobei* and the large *C. mastigophorus* also produced more in the Atlantic Ocean than in the Mediterranean Sea ($p<0.001$)(Fig. 6.20c). Despite *C. parapergens* reproductive parameters were only studied in spring 2002 in the Gulf of Naples, its secondary production in this area was similar to the lower measures obtained in *C. jobei* and *C. mastigophorus* (Fig. 6.20c). *C. arcuicornis* had significantly higher production in the Ionian Sea and in winter-spring in the Gulf of Naples ($p<0.001$)(Fig. 6.20b). Secondary production was highest but also very variable in *C. lividus* (max. 99.3 μg C m^{-3} d^{-1})(Table 6.19) which produced significantly more at the coastal station in the Gulf of Naples in winter-spring 2002 ($p<0.001$)(Fig. 6.20d). Only two clutches were observed in *C. ingens*, and its calculated secondary production was the lowest (Fig. 6.20d). The highest recruitment was estimated in *C. pergens* in the North Balearic Sea while the lowest were these of *C. mastigophorus* and *C. jobei* in the Gulf of Naples (Table 6.20). Due to high embryo viability, estimated recruitment in sac-spawners match with the estimated secondary production, while in broadcast spawners the estimated recruitment was lower than the estimated secondary production (Fig. 6.20).

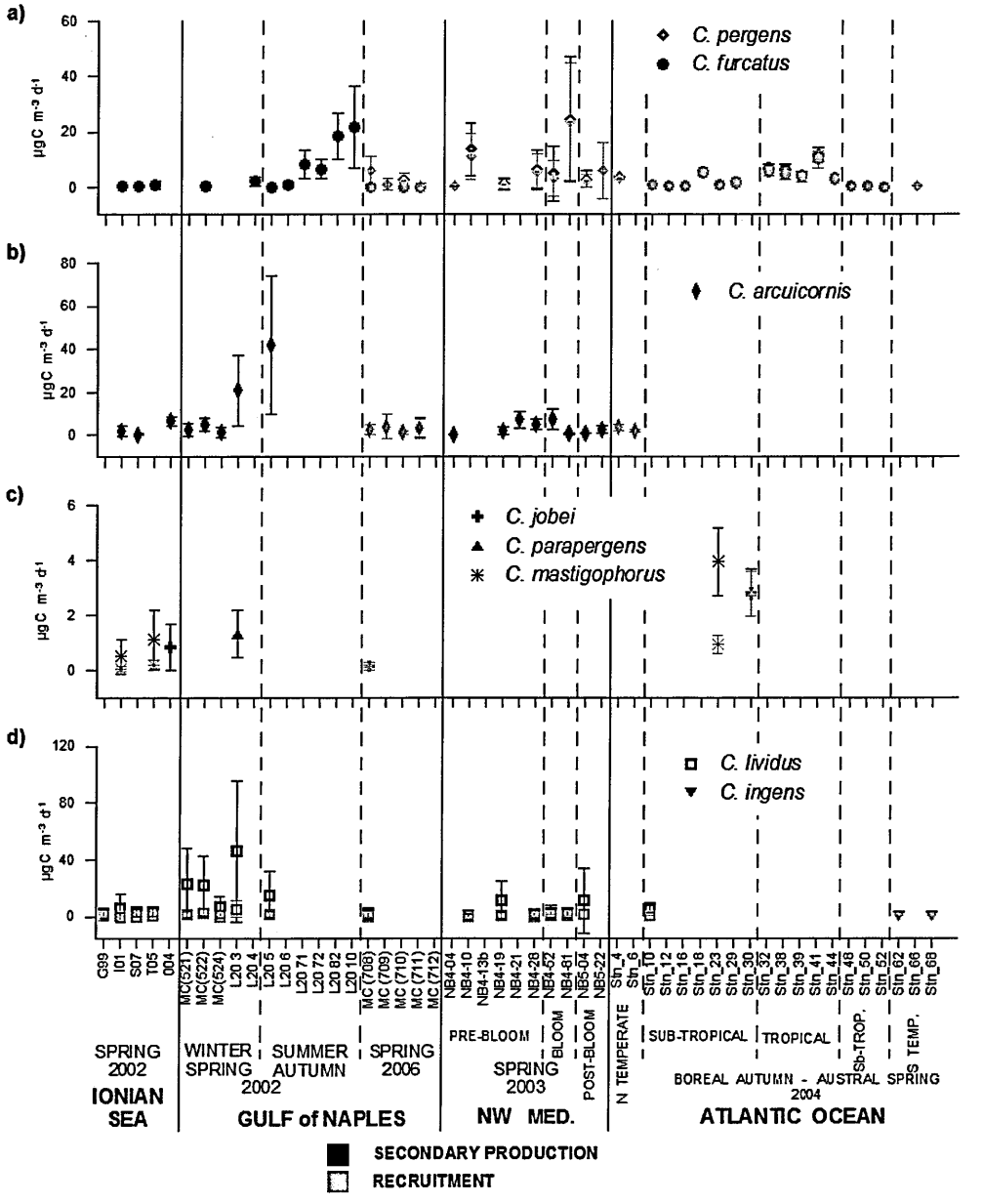

Figure 6.20. Secondary production of adult females (black) and recruitment (gray) of *Clausocalanus* species (both expressed in μg C m^{-3} d^{-1}). When both estimations were similar or equal, recruitment overlaps secondary production and only gray symbols can be observed. (a) *C. pergens* and *C. furcatus* (small-sized species, sac spawners), (b) *C. arcuicornis* and (c) *C. parapergens* and *C. jobei* (medium-sized species, sac spawners), and (d) *C. lividus*, *C. mastigophorus* and *C. ingens* (large-sized species, broadcasters).

Table 6.19. Secondary production of *Clausocalanus* species at each studied area (mean and range, μg C m^{-3} d^{-1}). Lines separate species in three size categories: small, medium and large, respectively.

Species	Ionian Sea	Gulf of Naples	NW Med.	Atlantic	TOTAL
C. pergens	-	1.77	6.70	1.93	3.87
		(0-11.2)	(0-41.9)	(0.2-4.4)	(0-41.9)
C. furcatus	0.43	5.60	-	2.56	3.85
	(0-2.5)	(0-40.9)		(0-14.7)	(0-40.9)
C. jobei	0.85	0.14	-	2.81	1.20
		(0-0.2)		(2.0-4.0)	(0-4.0)
C. parapergens	-	1.32	-	-	-
		(0.0-2.4)			
C. arcuicornis	3.67	9.18	3.14	3.09	6.30
	(0-9.9)	(0-75.5)	(0-10.9)	(1.2-6.1)	(0-75.5)
C. lividus	3.36	16.87	3.43	5.95	7.95
	(0-28.4)	(0-99.3)	(0-59.0)	(3.4-8.0)	(0-99.3)
C. mastigophorus	0.67	-	-	3.93	1.32
	(0-2.1)			(3.1-5.3)	(0-5.3)
C. ingens	-	-	-	0.25	-
				(0.2-0.3)	

Table 6.20. Recruitment of *Clausocalanus* species at each studied area (mean and range, μg C m^{-3} d^{-1}). Lines separate species in three size categories: small, medium and large, respectively.

Species	Ionian Sea	Gulf of Naples	NW Med.	Atlantic	TOTAL
C. pergens	-	1.75 (0-11.2)	5.77 (0-41.9)	1.87 (0-4.4)	3.50 (0-41.9)
C. furcatus	-	0.02 (0-0.2)	-	2.16 (0-14.4)	1.84 (0-14.4)
C. jobei	-	0.14 (0-0.1)	-	2.77 (1.9-3.8)	1.45 (0-3.8)
C. parapergens	-	-	-	-	-
C. arcuicornis	-	2.96 (0-15.7)	-	3.03 (1.2-6.1)	2.98 (0-15.7)
C. lividus	0.20 (0-3.7)	0.25 (0-13.7)	1.54 (0-10.1)	0.41 (0.4-1.3)	0.88 (0-13.7)
C. mastigophorus	0.77 (0-0.4)	0.10 (0.6-1.2)	-	-	0.95 (0.6-1.2)
C. ingens				0.27 (0.2-0.2)	

6.3. Discussion

The ecological success of a species in a given environment, depends on a fragile equilibrium of different factors (quantity and quality of proxy food, predation, parasitism, competition) but for sure the most important falls on its reproductive success. High reproductive success implies good reproductive performances (mating behaviour, egg laying, egg production rates, development times of eggs, growth rates and adult longevity) but also good survival rates (egg to adult viability) all along the twelve differentiated developmental stages of copepods.

In epipelagic calanoid copepods, two different spawning features occur. Most of the species are broadcast spawners and release the eggs freely in the water, while others are sac spawners, i.e. they carry the eggs in sacs or masses attached to female urosome. The former mode could be one of the results from the re-colonization by calanoid copepods of marine pelagic environments since sac-carrying females become more evident and suffer higher predation pressure than broadcast spawners (Webb and Weaver, 1988). Free spawned eggs risk being lost by sinking or predation, suffering one order of magnitude higher mortality rates than carried eggs (Kiørboe and Sabatini, 1994) even though ovigerous sac spawners females are more evident and vulnerable to visual predators (Vuorinen *et al.*, 1983; Bollens and Frost, 1991). Shorter egg hatching times in broadcasters compared to sac spawners may result from such strong selection pressure to reduce the length of this vulnerable period (Hirst and Kiørboe, 2002). On the other hand, females that carry the eggs cannot lay another clutch until eggs hatch (sac-spawned eggs take $\approx$1.9 times longer on average to hatch, according to Hirst and Kiørboe, 2002) and so broadcast-spawning copepods have higher weight-specific fecundities and egg production rates than sac spawners species (Kiørboe and Sabatini, 1995; Hirst and Bunker, 2003; Bunker and Hirst, 2004).

The occurrence of two different spawning features in *Clausocalanus* was observed only recently (Saiz and Calbet, 1999), after the revision of the biology of calanoid copepods conducted by Mauchline (1998), where similar cases are reported only for two other marine copepod genera: while *Euaugaptilus magnus* has an egg mass in the Rockall Trough (Mauchline, 1988), no other species in this genus have been observed to have one, and while *Chiridius gracilis* (a marine bottom-living genus) forms an egg mass (MacLellan and Shih, 1974), *C. armatus* does not (Matthews, 1964). Giesbrecht (1892) illustrated a female *Clausocalanus arcuicornis* carrying an egg-sac and this figure was copied by Rose (1933), but the presence of different reproductive features was never mentioned (Lindley, 1997). Nor in the generic or specific diagnostic features reviewed by Frost and Fleminger (1968), or in Frost (1969) who observed *C. paululus*, *C. furcatus*, *C. jobei*, *C. minor* and *C. farrani* bearing egg sacs in fixed samples, such differences in spawning features were reported. I noticed differences also among sac spawners: *C. arcuicornis*, *C. parapergens*, *C. jobei* and *C. pergens* carry an egg sac hanging from the genital pore, while *C. furcatus* carry the eggs wrapped at the urosome forming a mass. There is no evidence that the eggs are contained in a membrane in sac spawner calanoid copepods, but apparently the secretions that form the outer membrane of eggs seem to be more copious in egg mass carriers so that eggs as they are laid do not separate but stick in a mass attached to the genital pore (Mauchline, 1998). According to this definition, the term *sac spawner* is not correct in calanoid copepods but it has been the commonest nomenclature in the literature so it has been also used in this thesis. Actually, in *Clausocalanus* the presence of a thin and transparent membrane has been observed. Remains of material that surrounded the egg mass was observed in *C. jobei*, and reported by Mazzocchi and Paffenhöfer (1998) in *C. furcatus*.

In the large incubation volumes used during this study (300-2130 ml), females that laid a sac carried it until the nauplii hatched. It is true that egg sac and masses are quite fragile in *Clausocalanus*, but only when the ovigerous females were kept in stressful conditions, such as in low volumes of water (like in small crystallizers) or trapped in the air-water interface, eggs were rapidly released. Cornils (2005), Bi (2005) and Bi and Benfield (2006) reported the occurrence of free eggs in *C. furcatus*. Cornils (2005) incubated *C. furcatus* and *C. farrani* females individually in multiwells (5 ml) for egg production experiments so low incubation volumes and air-water interface could explain the occurrence of free eggs that she observed. Bi (2005) incubated groups of 5 females in large volumes (400 ml bottles) and observed that they easily discharged eggs when disturbed. Bi and Benfield (2006) incubated 5 females in 200 ml beakers which were aerated with small airstones. Both air-water interface and turbulence would likely make *C. furcatus* discharge the eggs mass. Sac release of sac spawning copepods was reported for *Sinocalanus tenellus* by Kimoto *et al.* (1986) who observed that about 20 eggs formed a mass attached to the ventral side of the genital somite but after a few minutes they separated freely into the water. The spawning of ribbons (or groups of eggs) was reported by Yang (1977) in *Parvocalanus crassirostris.* But neither of these features were observed in sac spawner *Clausocalanus* during the present thesis. Why eggs in *C. furcatus* are attached to the urosome while in the other *Clausocalanus* sac spawners hang from it? According to Bradford-Grieve (2002), Clausocalanoidea colonized several times the pelagic domain, so the sac-spawning feature could be an ancestral reminiscence from benthic living forms. The reconstruction of evolutionary relationships among *Clausocalanus* species based on the mtCOI type specific sequence Bucklin *et al.* (2003) revealed that *C. furcatus* was the ancestral species of the genus. Despite the relationship among the rest

of the species was not clarified by the genetic analysis, broadcast species were clustered together and were isolated from the sac spawners. Then, eggs in mass surrounding the urosome might be an ancestral reproductive feature that evolved to hanging eggs mass, and later to broadcast spawning, so changing from a more solid structure to a more fragile one to finally evolve to the unstructured free eggs laying behaviour.

Reproductive parameters of *Clausocalanus* have been reported so far for few species (Table 6.21). This is the first report of *C. parapergens* reproductive parameters. The clutch size ranges observed in the present study in the sac-spawners *C. furcatus*, *C. arcuicornis*, *C. jobei* and *C. pergens* fall in the ranges reported by Sazhina (1987) but I recorded larger maximum values for the last two species likely because they were measured in wider environmental range than in Sazhina (1987). On the contrary, fewer individuals of the broadcasters *C. mastigophorus* and *C. ingens* were observed during this thesis, measuring larger clutch size than that reported in Sazhina (1987). Maximum clutch size measured in the broadcaster *C. lividus* fall in the range of maximum clutch size reported by Saiz and Calbet (1999). *C. lividus* maximum clutch size was up to tenfold higher than that measured in the small species and doubled that of the medium-sized *C. arcuicornis* and *C. jobei*. The clutch size range measured in the medium-sized sac-spawner *C. parapergens* fall in the range of the large broadcasters *C. mastigophorus* and *C. ingens*. Clutch size observed in both sac and broadcast-spawners *Clausocalanus* species fall in the range of similarly sized calanoids, even for the maximum values measured in *C. parapergens* (Mauchline, 1998, Tables 45, 46).

Egg production rates observed for *C. arcuicornis*, *C. furcatus*, *C. jobei*, *C. mastigophorus* and *C. ingens* during this thesis fall in the range reported by Sazhina (1987) except those observed for *C. pergens*, which were lower. The EPR here

reported for *C. lividus* fall in the maximum EPR values reported for *C. lividus* in winter in the western Mediterranean (Calbet *et al.*, 2002). EPR of broadcasters *Clausocalanus* were 3.7 times higher than that of sac-spawners, larger difference than that reported by Kiørboe and Sabatini (1995).

Since the interclutch period observed for the species of this genus is on most occasions quite long in natural conditions (also observed by Sazhina, 1987; Webber and Roff, 1995) as well as in laboratory conditions (also observed by Mazzocchi and Paffenhöfer, 1998), EPR calculations are affected by the maximum individual incubation time achieved during the experiments and has been overestimated due to the used methodology (see Section 6.1). In sac spawners species, interclutch period depends on embryo development time and hatching of nauplii. But in free spawners, interclutch period only depends on oogenesis and the spawning time, so interclutch period is expected to be shorter. Instead of that, broadcasters *Clausocalanus* had similar or even longer interclutch periods than its sac-spawners congeners. Differently from other broadcaster copepods that produce continuously, *Clausocalanus* spawn large amount of eggs in one single event, as if they retained the ancestral sac-spawning feature. In terms of carbon, the clutch could represent maximum 25% of female body carbon in sac-spawners while up to 75% in broadcast spawners indicating a maximum of carrying capacity in sac-spawners. In field conditions, the shortest interclutch period was observed in *C. furcatus* in mesotrophic environment (around the NW African upwelling) where the quantity and quality of food would be excellent for the egg production of this species. This findings contrast with those of Mazzocchi and Paffenhöfer (1998) who observed that *C. furcatus* egg production rate was higher at low food concentrations in laboratory conditions. Higher fluorescence values encountered in the NW African upwelling region could not necessary related to high concentration on food items.

On the other hand, natural diet is much more diverse than that offered under laboratory conditions covering much better the metabolic needs and would enhance higher egg production rates.

Despite that EPR of broadcast and sac spawner copepods is generally estimated over a single 24 h interval (Runge and Roff, 2000), some authors have criticized this method in sac spawner species arguing that such species typically produce clutches of eggs less constantly and then carry the same clutch for several days (Nielsen *et al.*, 2002). Sac-spawning species include all cyclopoids, poecilostomatoids, and harpacticoids plus the important calanoid genera *Pseudocalanus*, *Euchaeta* and *Clausocalanus*, which combined constitute a significant fraction of marine copepods. All *Clausocalanus* females incubated during the reproduction experiments here presented were incubated as long as possible and so the EPR was calculated according to the whole incubation period. In fact, when only the first 24 h were considered due to absence of new food supply (Gulf of Naples 2002), the largest EPR and weight-specific fecundity rates were recorded for both sac-spawners (*C. parapergens*) and broadcasters (*C. lividus*).

The present data on embryo viability are the first obtained for this genus. Embryo viability of sac-spawners *Clausocalanus* species was on average very high in comparison of that observed in the congeneric broadcasters. Embryo viability did not depend on re-mating, because eggs were fertilized even in successive clutches. This suggests that re-mating is not necessary in this genus (either in sac or broadcast spawners). On the other hand, embryo viability in *C. lividus* was 100% when was assessed with vital probes, suggesting that incubation time of eggs to assess their natural hatching success might be too short despite being 72 h as suggested by Runge and Roff (2000). Low embryo viability is expected in wild conditions during periods of scarce male:female sex ratio (due to lack of

fertilization) or due to diet toxic effects (Ianora *et al.*, 1996, but Irigoien *et al.*, 2002), while under laboratory conditions could be also due to a nutritive deficit by a mono-algal diet. Lower embryo viability observed in *C. furcatus* during April 2004 (season of minimum adult population) would be a consequence of such scarce male:female sex ratio, but no fertilization test was done.

Diet did not affect females clutch size and embryo viability, but it did impact EPR in *C. arcuicornis* and *C. lividus*, when the same set of females were incubated 48 h in natural particle assemblages and then in *Prorocentrum micans*. Concentration of the offered culture was a compromise between the minimum requirements of *C. furcatus* in tropical oligotrophic waters and the natural availability in the area at that time. Significant increase in EPR of *C. arcuicornis* and decrease in EPR of *C. lividus*, might reflect an increase of food availability for the medium-sized *C. arcuicornis* but not for the large *C. lividus* which could need larger carbon amount.

Embryo viability was compromised in *C. arcuicornis* when the sac was detached from the female at an early developmental stage. Evidence of maternal control of embryogenesis through chemical communication have been provided for various crustaceans as well as for a marine harpacticoid copepod (*Tigriopus japonicus* Kahan *et al.*, 1988). Such control, called 'umbilical cord' mechanism, can be directly due to environmental cues suffering the mother (e.g. food availability or quality, density of the conspecific population, the degree of hydrodynamic disturbance, predatory pressure, etc. Lonsdale *et al.*, 1998). The control of naupliar development would be advantageous in *C. arcuicornis* which rapidly increases its population in spring like triggered by the increase of food availability in that period. Mazzocchi and Paffenhöfer (1998) also noticed that *C. furcatus* eggs isolated from the mother never hatched even when well developed embryos could be ob-

served inside the eggs. This species also has a pronounced peak of abundance in summer above the thermocline like triggered by the increase of water temperature and/or the absence of congeners. On the other hand, the presence of already hatched nauplii in entire egg-sac of *C. jobei* might seem likely that the movements of the mother play an important part in helping the nauplii to hatch, as was observed in *Euchaeta norvegica* (Marshall and Orr, 1954). The normal swimming behaviour of *C. furcatus* in absence of turbulence is characterized by a repetitive looping at high speed (10 mm s^{-1} on average), with frequent changes of direction (Mazzocchi and Paffenhöfer, 1999) and is similar in all the other *Clausocalanus* species even in egg-bearing females (personal observation). When an egg-bearing female is stressed, it rapidly discharges the eggs likely due to the lash of the abdomen to quickly escape. So, in my opinion, *Clausocalanus* egg-sac is not fragile (as suggested by Mazzocchi and Paffenhöfer, 1998) and requires the involvement of the mother to break out. The 'umbilical cord' mechanism does not rule out the involvement of the mother to break out the sac and vice versa, but further investigation is required.

Egg diameter, clutch size and clutch volume increased with body volume in *Clausocalanus* species, as occurs in other calanoids (Mauchline, 1998). Species females body length range fit with those reported by Frost and Fleminger (1968), except for *C. arcuicornis* and *C. lividus* in which smaller and larger specimens (respectively) were observed during the present study in all the areas (*C. arcuicornis*) or only in the North Balearic Sea (*C. lividus*). Williams and Wallace (1975) also observed smaller *C. arcuicornis* and larger *C. lividus* than that reported by Frost and Fleminger (1968). While *C. arcuicornis* measurements were smaller than that reported by Williams and Wallace (1975), those of *C. lividus* were almost as large as that reported by the authors. Estimated carbon contents of *C. fur-*

catus was similar to that estimated from ash-free dry weight measures performed by Hopcroft and Roff (1998), Mazzocchi and Paffenhöfer (1998), and Paffenhöfer *et al.* (2006). Estimated carbon contents of other *Clausocalanus* species (*C. pergens*, *C. furcatus*, *C. jobei*, *C. arcuicornis*, *C. mastigophorus* and *C. ingens*) were in the range reported by Sazhina (1987) although I could not find how this author obtained such measurements as methods were not detailed. Regarding eggs diameter, those measured for *C. furcatus* were in the range reported by Mazzocchi and Paffenhöfer (1998) (50-78 μm) and Hopcroft and Roff (1998) (79 μm) while those measured for *C. lividus* were larger than that reported by Saiz *et al.* (1999) (66-71 μm). Sazhina (1987) reported similar egg diameters ($\approx$ 70 μm) for five species (*C. pergens*, *C. furcatus*, *C. jobei*, *C. arcuicornis*, *C. mastigophorus*) except for *C. ingens* ($\approx$ 50 μm).

Mean weight-specific fecundity rates observed in *Clausocalanus* broadcast spawners were higher than the general mean reported by Hirst and Bunker (2003) for this reproductive mode while in *Clausocalanus* sac spawners rates were lower than the general mean. In the general observed patterns, Hirst and Bunker (2003) observed higher WSF in broadcasters than in sac spawners while in *Clausocalanus* the opposite was observed mainly due to the influence of body weight since WSF increase with body weight and in *Clausocalanus* only the larger species are broadcasters while the small ones are sac spawners.

Weight-specific fecundity rates vs. temperature regression models improved when the analyses were conducted at species level rather than at spawning mode or at genus level, highlighting that species have different relationship with the environmental parameters and that gathering information of different spawning modes would result in a loss of resolution. Negative relationships were observed at the genus level, mainly due to sac spawners while broadcast spawners showed

positive relationship with this parameter. Since the study of species egg production rates was limited to areas where species population was higher, the studied temperature range was narrower than in laboratory experiments and in review papers reporting both positive and negative relationship between WSF and temperature according to how many measurements were performed below or above the optimum temperature of the species. The Q_{10} temperature coefficient is a measure of the rate of change of a biological or chemical system as a consequence of increasing the temperature by 10°C. The Q_{10} observed in *Clausocalanus* were in the range of that reported by Hirst and Bunker (2003)(Q_{10} 1-2) except for *C. arcuicornis* and *C. lividus*. Those temperature coefficients were more similar to those observed in copepods reared in food saturated conditions (laboratory)(Q_{10} 2.5-4) rather than that observed *in situ*, suggesting that these species were mainly studied in their optimum trophic conditions.

Scatter-plots of weight-specific fecundity rates against Chl *a* concentration suggested that there was no a functional response of weight-specific fecundity rates with Chl *a*, confirmed when Michaelis-Menten (or Ivlev) curves did not fit. This kind of curve assumes an accelerating weight-specific fecundity rate with increasing concentrations of Chl *a* reaching a threshold when minimum Chl *a* concentration reports maximum weight-specific fecundity rate. The lack of relationship was not surprising since *Clausocalanus* is not a strict herbivorous genus (Kleppel, 1993) specially in oligotrophic environments where mesozooplankton community graze on intermediate consumers (Calbet and Landry, 1999). Copepods feeding on microzooplankton may have high production rates, unrelated to Chl *a* concentrations (Gifford and Dagg, 1988) probably due to varied diet. *Clausocalanus* and *Oithona* dominate the copepod assemblage in oligotrophic environments and they perform very different and particular feeding behaviour but

both graze on microzooplankton (Paffenhöfer and Mazzocchi, 2002). Hirst and Bunker (2003) observed that in *Oithona* spp. some high weight-specific fecundity values at very low Chl *a* values also disarranged Michaelis-Menten curves. For these groups, Chl *a* concentration is therefore not a good measure of quantity and quality of available food. Walker and Peterson (1991) suggested that it might be more appropriate to relate primary productivity rather than simply phytoplankton biomass to secondary productivity.

Multiple regression analyses of WSF vs. both environmental parameters and body weight revealed that in the sac-spawners *C. pergens* both temperature and Chl *a* concentration (positively related) were explicative for its WSF rates, in *C. jobei* only Chl *a* concentration (negatively related), in *C. furcatus* and *C. arcuicornis* all three parameters (temperature and body weight negatively in *C. furcatus* and positively in *C. arcuicornis* and Chl *a* positively for both species). In the broadcast spawner *C. lividus* both Chl *a* and female body weight (negatively related) but not temperature.

Secondary production in *Clausocalanus* was similar to values reported for several calanoid copepods in the on-shore and open waters in Oregon (Peterson *et al.*, 2002). *Clausocalanus* species showed clear peaks of mean secondary production occurring at different sites in accordance to the species environmental preferences. *C. pergens* highest secondary production was estimated in the NW Mediterranean during the bloom occurrence, while *C. furcatus* secondary production peak occurred in summer at offshore waters in the Gulf of Naples. *C. jobei* peak occurred in the Canary current region and that of *C. parapergens* and *C. arcuicornis* in spring at the offshore site in the Gulf of Naples. *C. lividus* peak also occurred in spring at the offshore site in the Gulf of Naples while that of *C. mastigophorus* occurred in the Canary current. Despite *C. lividus* was among the least

abundant species, its secondary production was comparable to that of the sac spawning *C. arcuicornis* and twofold times higher than the most abundant small species *C. pergens* and *C. furcatus*. Nevertheless, estimated nauplii recruitment expressed in terms of carbon concentration (μg C m^{-3} d^{-1}) was similar or equal to the species secondary production in sac-spawners, while it was lower in broadcast spawners due to low measured hatching success. In *C. lividus*, despite its large secondary production, its recruitment was low similar to that of the least abundant *Clausocalanus* species *C. jobei*, *C. parapergens* and *C. mastigophorus*, which would limit its population abundance. It has been seen (Chapter 2) that sex ratio (one of the principal factors limiting egg fertilization in copepods according to Kiørboe, 2007) in *Clausocalanus* broadcast spawners was higher than that of the congeneric sac spawners, thus fertilization rate would not limit the population growth rate. Low hatching rates were reported by Irigoien *et al.* (2002) in significant fractions of mature copepod females in field conditions plenty of food. Vital probes revealed that *C. lividus* eggs were fertilized in spring in the Gulf of Naples and that apparently they were viable; nevertheless during reproduction experiments without dyes observed hatching success was very low.

In synthesis, egg hatching success, embryo viability and egg fertility were measured for the first time in this genus. Egg fertility in successive clutches suggested that re-mating is not necessary in the species of this genus. Embryo viability was high in sac-spawners while hatching success was very low in broadcast spawners compromising their recruitment since secondary production was comparable among the two spawning modes. Clutch size, egg production rate and weight-specific fecundity increased with body weight. Weight-specific fecundity rates were related to those environmental parameters that also characterized species optimum environmental conditions of occurrence. Secondary production

estimated on the basis of weight-specific fecundity was higher in one broadcast species but not for the other. Secondary production was not always related to species population abundance or spawning modes.

Table 6.21. Studies on *Clausocalanus* species reproductive parameters published after the revision of Frost and Fleminger (1968). Abbreviations: *n* number of individuals observed; *CS* clutch size (number of produced eggs); *EPR* egg production rate (eggs f^{-1} d^{-1}); *IP* interclutch period (days); *WSF* weight-specific fecundity (d^{-1}); *NPA* natural particle assemblage; *Temp* temperature; *Medit* Mediterranean Sea.

Author	*Species*	n	CS	EPR	IP	WSF	Diet	Temp.	Site
Mazzocchi and	*C. furcatus*	2	6.5±0.5				*Rhodomonas*	20	Atlantic
Paffenhöfer, 1998	*C. furcatus*	19	17.2±8.5				*Gymnodinim nelsoni*	20	Atlantic
	C. furcatus	4	17.5±10.3				*Talassiosira weissflogii*	20	Atlantic
	C. furcatus				1-2				Atlantic
Saiz and Calbet, 1999	*C. lividus*		133-259				NPA		Medit.
Saiz et al., 1999	*C. lividus*	5		10.9 (SE 9.0)		0.027 (SE 0.010)	NPA		Medit.
	C. lividus	5		7.2 (SE 2.0)		0.016 (SE 0.003)	NPA		Medit.
	C. lividus	7		2.2 (SE 2.0)		0.005 (SE 0.002)	NPA		Medit.
Calbet et al., 2002	*C. lividus*	12-20		35.0 (SE 15.0)		0.050 (SE 0.020)	NPA		Medit.
	C. lividus	12-20		25.0 (SE 20.0)		0.020 (SE 0.190)	NPA		Medit.
	C. lividus	12-20		15.0 (SE 10.0)		0.030 (SE 0.016)	NPA		Medit.
	C. lividus			5.0 (SE 5.0)		0.010 (SE 0.004)	NPA		Medit.
	C. lividus			5.0 (SE 5.0)		0.010 (SE 0.005)	NPA		Medit.
	C. lividus			10.0 (SE 5.0)		0.020 (SE 0.005)	NPA		Medit.
	C. lividus			100 (max.)			NPA		Medit.
Bi and Benfield, 2006	*C. furcatus*	60	12-18	12.1 (SE 1.4)			NPA	18-28	Atlantic
Cornils et al., 2007	*C. furcatus*	30-90	1.5-13.6	1.8-9.2			NPA	22-27	Indian
	C. farrani	30-90	2.7-9.8	0-3.3			NPA		Indian

Table 6.21 (Continued)

Author	Species	n	CS	EPR	IP	WSF	Diet	Temp.	Site
Sazhina, 1985	*C. furcatus*		16-22				NPA		Indian
Sazhina, 1987	*C. arcuicomis*	22	62±46.7	62.0	1	0.008	NPA	19	Atlantic
(Tables 10-11, 13-14)	*C. arcuicomis*		34±15.1	34.0	1	0.006	NPA	16	Atlantic
	C. arcuicomis		19±2.3	19.0	1	0.003	NPA	12	Atlantic
	C. arcuicomis	1	25	25.0	1	0.005	NPA	15-17	Medit.
	C. arcuicomis	5	40	40.0	1	0.008	NPA	18-22	Medit.
	C. arcuicomis	5	28	56.0	0.5	0.011	NPA	23-25	Medit.
	C. furcatus	17	27±8.7	9.0	3	0.002	NPA	21	Atlantic
	C. furcatus		61±19.5	20.3	3	0.006	NPA	12	Atlantic
	C. furcatus		19±2.1	6.1	3	0.001	NPA	19	Atlantic
	C. furcatus	3	35	11.6	3	0.002	NPA	18-22	Medit.
	C. ingens	15	49±12.3	49.0	1	0.003	NPA	15	Atlantic
	C. jobei	7	24±7.4	8.1	3	0.002	NPA	20	Pacific
	C. mastigophorus	12	19±11.2	19.0	1	0.003	NPA	14	Atlantic
	C. pergens	3	23±2.5	11.5	2	0.002	NPA	24	Atlantic
Webber and Roff, 1995	*C. furcatus*		15		3.3				Atlantic
Hopcroft and Roff, 1998	*C. furcatus*			4.5		0.080	NPA		Atlantic

CHAPTER 7

Overall scenario

This thesis focused on the study of the ecology and reproductive biology of *Clausocalanus*, a planktonic calanoid genus that is widespread both in oceanic and coastal marine waters but still poorly investigated. The research has been in particular aimed at characterizing the niches of those *Clausocalanus* species occurring in Mediterranean and Atlantic waters for a better understanding of their success in various marine regions.

Niches were characterized according to the two major environmental factors affecting the distribution and the biological performance of the epipelagic planktonic community: temperature (affecting metabolism and development) and fluorescence (the proxy for autotrophic biomass and considered representative of potential food available); and to the three dimensional spatial axis observed in this study (vertical, horizontal and latitudinal distribution).

The obtained results show that *Clausocalanus* was present and numerically important in all the investigated regions both in the Mediterranean Sea and in the Atlantic Ocean where up to eight of its species co-occurred in most of these sites. However, although most *Clausocalanus* species did widely co-occur over spatial and temporal scales, the characteristics of their distribution could be clearly discerned, which, coupled with species reproductive performances, allowed to depict species niches and evaluate the degree of their niche separation. Among the 13 *Clausocalanus* species reviewed by Frost and Fleminger (1968), 11 species occur in the Atlantic and 8 of them in the Mediterranean. *C. paululus*, *C. pergens*,

C. furcatus, *C. jobei*, *C. parapergens*, *C. arcuicornis*, *C. lividus* and *C. mastigophorus* were present in most of the surveyed sites, while *C. ingens*, *C. brevipes* and *C. laticeps* were restricted to the southern Atlantic Ocean.

Clausocalanus assemblages were dominated by the small-sized species *C. paululus*, *C. pergens* and *C. furcatus* both in the Mediterranean Sea ($74.5 \pm 20.4\%$) and in the Atlantic Ocean ($85.4 \pm 18.1\%$). They were followed by the medium-sized species *C. jobei*, *C. parapergens*, *C. arcuicornis*, *C. brevipes* and *C. laticeps*, the first three making up $19.8 \pm 18.0\%$ in the Mediterranean and all five $13.2 \pm 18.1\%$ in the Atlantic. The less representative were the large-sized species *C. lividus*, *C. mastigophorus* and *C. ingens*, the fist two acounting for $5.6 \pm 7.9\%$ in the Mediterranean Sea and all three species only $1.4 \pm 1.9\%$ in the Atlantic Ocean.

The small-sized *Clausocalanus* species showed clearly defined niches (Fig. 7.1):

- *C. paululus* and *C. pergens* were widely distributed on the temporal and spatial (vertical and latitudinal) scales, while *C. furcatus* was restricted to summer-autumn and to the upper layer;

- All three species had wide thermal niches breath but *C. paululus* and *C. pergens* preferred cold waters while *C. furcatus* preferred warm waters;

- Despite *C. paululus* and *C. pergens* occurred over a wide trophic niche breath, *C. paululus* occurred with similar abundances either in eutrophic or oligotrophic conditions while *C. pergens* peak abundances were more restricted to eutrophic waters. *C. furcatus* had narrower trophic niche breath, occurring in oligotrphic-mesotrophic waters.

- So *C. paululus* had the widest realized niche occurring in cold-temperate oligotrophic and eutrophic conditions, *C. furcatus* had a narrower realized niche occurring in warm oligotrophic-mesotrophic conditions, while *C. per-*

gens had the narrowest realized niche restricted to cold eutrophic waters.

- When *C. pergens* and *C. paululus* realized niches overlapped, they co-exist but the former outnumbered the latter. *C. furcatus* realized thermal niche almost did not overlap with those of *C. pergens* and *C. paululus*. When they co-existed, *C. furcatus* occurred at very low abundance. In integrated samples from large vertical range, these three species seemed to co-exist (e.g. in the Atlantic Ocean) while the study at finer scale (offshore station in the Gulf of Naples) revealed that they were separated in the vertical plane, *C. furcatus* occurring in the warm surface waters and the other two in deeper colder waters;

- *C. pergens* and *C. furcatus* had similar secondary production but their maximum productions were recorded in opposite thermal conditions: *C. pergens* had its maximum in cold eutrophic conditions while *C. furcatus* had it in warm mesotrophic conditions, reinforcing the large divergence of *C. pergens* and *C. furcatus* realized niche mainly on the thermal component.

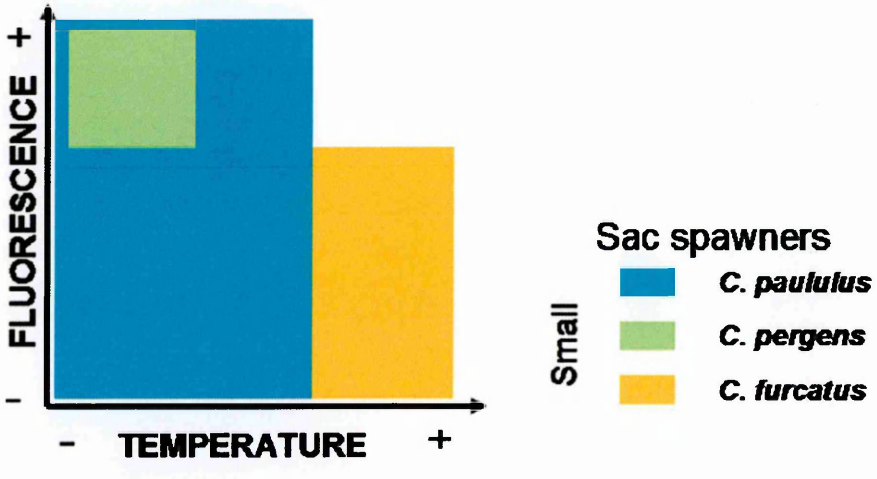

Figure 7.1. Schematic representation of small *Clausocalanus* species' niche on the thermal and the trophic components.

Niche characterization of medium-sized *Clausocalanus* species was less clear (Fig. 7.2):

- *C. jobei*, *C. parapergens* and *C. arcuicornis* had similar horizontal and latitudinal distributions, as well as similar seasonal cycles suggesting largely overlapping niches;
- *C. jobei* showed high onshelf preferences while *C. arcuicornis* was more oceanic but it does succeed both in coastal and oceanic environments;
- *C. parapergens* realized niche was characterized by oligotrophic colder conditions than *C. arcuicornis* and *C. jobei*, showing vertical differentiation from these two species during the overlapping populations peak (in spring at the offshore station in the Gulf of Naples);
- *C. arcuicornis* generally outnumber the other two species except at onshore stations in the Atlantic Ocean where *C. jobei* dominated;
- Secondary production of *C. jobei* and *C. parapergens* was one order of magnitude lower than that of *C. arcuicornis*;
- *C. arcuicornis* maximum secondary production occurred in spring in the Gulf of Naples, suggesting that despite this species had a large niche breath, its fitness was higher in mesotrophic conditions. *C. jobei* maximum secondary production was measured in the Canary region, reinforcing that *C. jobei* realized niche was not only characterized by temperate mesotrophic conditions but also by its neritic preference.

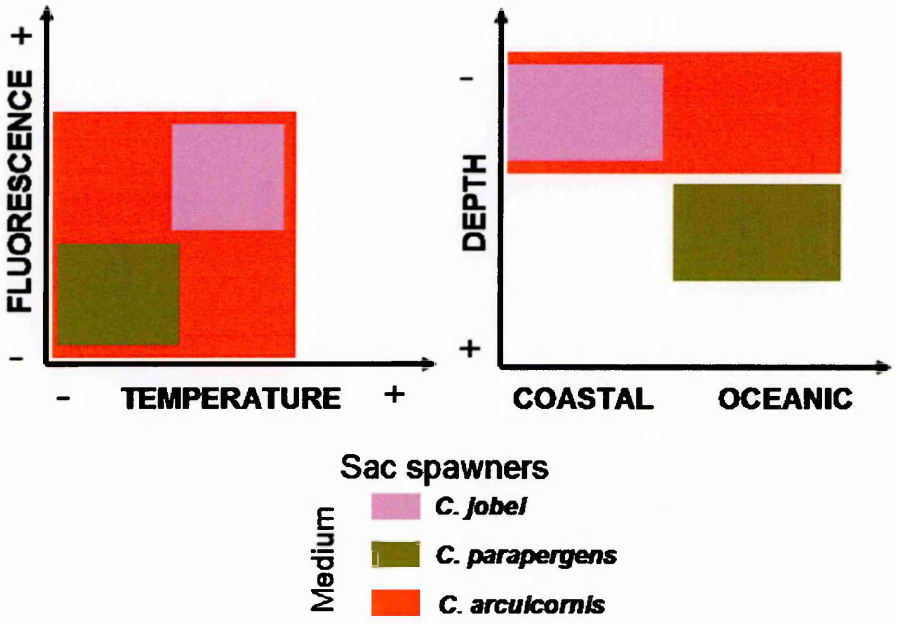

Figure 7.2. Schematic representation of small *Clausocalanus* species' niche on the thermal and the trophic components and on the spatial (depth in the water column and distance to coast) components.

Regarding the large-sized *Clausocalanus* species, their niches could be characterized despite the low population abundance observed (Fig. 7.3):

- *C. lividus* trophic niche breath extended from oligotrophic to eutrophic conditions while that of *C. mastigophorus* did not extended into eutrophic conditions.

- The thermal component of the niche highly differed among the two species: *C. lividus* occurred in temperate antitropical waters while *C. mastigophorus* in warm waters;

- Secondary production estimates reinforced that *C. lividus* and *C. mastigophorus* would overlap on the trophic component of the niche in oligotrophic-mesotrophic waters but they were separated by the thermal component.

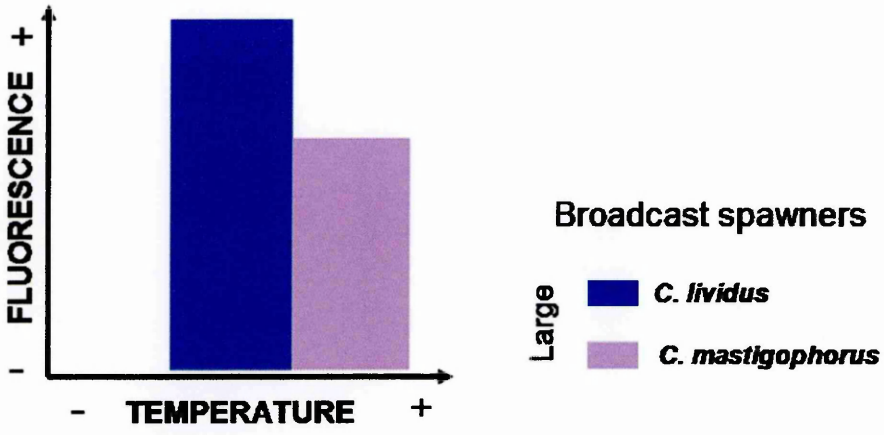

Figure 7.3. Schematic representation of large *Clausocalanus* species' niche on the thermal and the trophic components.

Niche overlap among species of different size was also observed:

- *C. paululus*, *C. pergens*, *C. arcuicornis* and *C. lividus* in eutrophic oceanic waters but *C. pergens* outnumbered the other species;
- *C. pergens* and *C. jobei* in cold coastal eutrophic conditions and none of them outnumbered the other;
- *C. paululus*, *C. arcuicornis* and *C. lividus* in cold oceanic oligotrophic waters but *C. paululus* outnumbered the others;
- *C. furcatus* and *C. mastigophorus* in warm waters but the former outnumbered the later.

The observed co-occurrences could suggest that inter-species differences in body size could also imply differences in mouthparts (size and/or intersetula distance or length) as well as differences in swimming behaviour resulting in different feeding performance that would allow species to co-occur. The few works that have focused so far on *Clausocalanus* feeding and swimming behaviour (see Table 1.2) considered only a few species (*C. pergens*, *C. furcatus*, *C. arcuicornis*,

C. farrani, *C. laticeps*, and *C. lividus*) and comparative analyses cannot yet be performed to test this hypothesis. The degree of size differences required to avoid feeding competition is largely discussed (Frost, 1980; Simberloff and Boecklen, 1981; Losos *et al.*, 1989) and competition cannot be dismissed.

When the two different egg-laying modes were analysed, interesting observations rise up:

- Small and medium-sized species are sac-brooders while large species are broadcast spawners;
- Clutch size increases with females size, so different egg-laying modes could be consequence of predation pressure;
- In *Clausocalanus*, only the smallest sac-spawners were the most abundant species (*C. paululus*, *C. pergens*, *C. furcatus* and *C. arcuicornis*) while *C. jobei* and *C. parapergens* were among the least abundant together with the broadcast spawning species;
- Broadcast spawners showed the highest weight-specific fecundity rates of the genus but showed similar secondary production to sac-spawners despite they never occurred at high abundance;
- Low population abundance of the broadcast spawning species could be probably related to their low recruitment which was estimated as the lowest among congeners due to low egg hatching success;
- Re-mating did not seem to be necessary in either of the two different egg-laying modes since stained embryos nuclei of successive clutches indicated that eggs were fertilized despite the isolation of the females.

In conclusion, the niches of eight coexisting *Clausocalanus* species have been characterized. Niches were clearly separated among congeneric species that were similar in size but showed a large degree of overlapping in congeneric species that differed in size, suggesting that size might be sufficient to prevent competition, or congeneric niches would be subtlety separated by environmental factors or biological traits that have not been considered in the present study. Species reproductive parameters and egg-laying mode largely account to shaping up the species niche and to examine their population success throughout secondary production and recruitment estimates. The large occurrence and degree of niche separation of the three small-sized *Clausocalanus* species point them as good indicators of water masses and global climate change. These results supply a consistent evidence of niche separation among similar in size congeneric species in pelagic ecosystems, sustaining the ecological and biological differentiation of species to allow coexistence.

The present thesis supplies new quantitative data on eight *Clausocalanus* species not only on adult females but also on adult males over a large distribution range both in the Mediterranean Sea and in the Atlantic Ocean. New data on reproductive features and parameters, including for the first time egg hatching rate and embryo viability, this last assessments based on a novel method that has been set up during this thesis, allowed the first secondary and recruitment estimates for seven species. By integrating all the ecological and biological data, species niches were depicted and the extent of niches separation was evaluated.

This thesis represents an integrated contribution to our knowledge of the ecology and biology of *Clausocalanus*, and, hopefully, a stimulus for addressing still open questions on this very interesting copepod genus.

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